

DETERMINATION OF THE VITAMIN B-6 REQUIREMENT
OF PREGNANT WOMEN

By

KAREN SCHUSTER

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To Glenn and his father Gordon,
for their love of learning

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INTRODUCTION

The 1980 Recommended Dietary Allowance (RDA) for vitamin B-6 for pregnant women is extrapolated from depletion-repletion studies with nonpregnant women. The increase in the RDA during pregnancy from 2 mg to 2.6 mg is based on the additional vitamin B-6 needed for the increased protein allowance. No additional vitamin B-6 is recommended to compensate for fetal demand, increased maternal metabolic requirements, and hormonal induction of maternal vitamin B-6 dependent enzymes.

Numerous studies have demonstrated that pregnant women have significantly lower blood vitamin B-6 levels, plasma pyridoxal 5'-phosphate (PLP) levels, and decreased erythrocyte aminotransferase activities as well as decreased PLP saturation of these enzymes compared with nonpregnant women. Plasma PLP levels of pregnant women have been reported to be very low at term, with a significant number having levels indicative of a vitamin B-6 deficiency relative to nonpregnant women. Several vitamin B-6 supplementation trials have shown that supplementing pregnant women with 2 to 4 mg of vitamin B-6 is insufficient to maintain normal biochemical indices of vitamin B-6 status throughout pregnancy.

These supplementation trials have not controlled for prior supplementation with vitamin B-6 or oral contraceptive use, and little attempt to quantify dietary vitamin B-6 intake has been made. These studies have indicated that the current vitamin B-6 RDA for pregnant women is inadequate to maintain normal biochemical indices of vitamin B-6 status, and a carefully designed study with pregnant women receiving a placebo or graded doses of pyridoxine is needed to determine the vitamin B-6 requirement of pregnant women.

The objectives of this study were

1. to estimate the requirement for vitamin B-6 of pregnant women,
2. to examine the effects of graded doses of vitamin B-6 taken by pregnant women on the biochemical indicators of vitamin B-6 status of the mothers at the 30th week of pregnancy and at delivery and of the infant at birth,
3. to assess the the effects of graded levels of maternal vitamin B-6 supplementation on the condition of the infants at birth,
4. to examine the relationship between vitamin B-6 status and the degree of morning sickness experienced by pregnant women during early pregnancy, and
5. to investigate the incidence of gestational diabetes and pre-eclampsia as related to vitamin B-6 status in a group of pregnant women.

Pregnant women attending Maternity and Infant Care clinics in north central Florida were randomly assigned

supplements containing 0 (placebo), 2.6, 5, 7.5, 10, 12.5, 15 or 20 mg of pyridoxine-HCl at their first prenatal appointment. Maternal health and vitamin B-6 status as measured by plasma pyridoxal 5'-phosphate levels and erythrocyte aspartate aminotransferase activity and stimulation by exogenous PLP were determined at the first clinic visit, 30 weeks gestation, and at term. The vitamin B-6 status and condition of the infant at birth were also assessed.

LITERATURE REVIEW

Vitamin B-6 History

In 1934 Gyorgy (1) differentiated the "rat pellagra preventative factor" from riboflavin (vitamin B-2) in rice bran and called the new vitamin "B-6." Five groups of scientists independently isolated the crystalline compound in 1938 (2-6). Its chemical structure was subsequently characterized, and the vitamin was synthesized by Harris and Folkers in 1939 and named pyridoxine (7).

The existence of other naturally occurring compounds which exhibited vitamin B-6 activity was revealed in microbiological studies (8, 9). In 1944 these forms of vitamin B-6 were identified as pyridoxal and pyridoxamine by Snell (10, 11) and synthesized by Harris et al. (12). Subsequent work indicated that vitamin B-6 functioned as a coenzyme in the phosphorylated form of pyridoxal (13).

Vitamin B-6 Chemistry, Absorption and Bioavailability

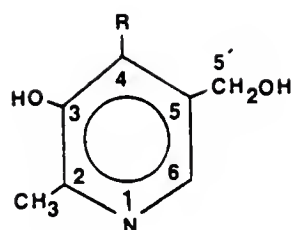
Vitamin B-6 is the recommended generic name for all 3-hydroxy-2-methylpyridine derivatives which exhibit the same biological activity. These compounds, pyridoxine, pyridoxal and pyridoxamine and their phosphorylated

derivatives, are shown in figure 1. Occurring as white crystals, the B-6 vitamers are water-soluble but less soluble in alcohol and often insoluble in ether. Pyridoxamine and pyridoxine hydrochlorides are stable in hot dilute mineral acid and alkali, but pyridoxal hydrochloride is unstable in basic aqueous solutions. All forms are extremely sensitive to ultraviolet as well as visible light (14-17) and destroyed by strong oxidizing agents (3, 14).

Vitamin B-6 appears to be absorbed by passive diffusion as indicated by a linear relationship between vitamin B-6 dosage and urinary excretion in human and animal studies (18-20). Recent evidence that rat jejunal uptake of pyridoxine (0.01 μ M - 10 mM) is directly proportional to its concentration supports this proposed mechanism (21).

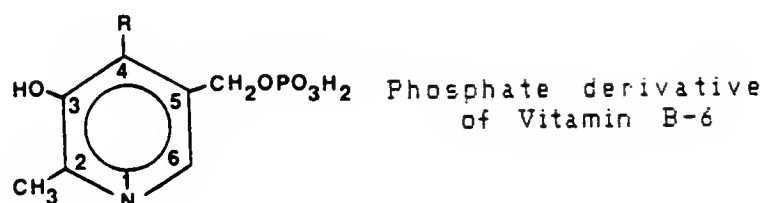
Absorption of tritium-labelled pyridoxine in rats occurs rapidly from the upper intestine and slightly less rapidly from the ileum (22). Some absorption from the colon but none from the stomach was observed when the pyridoxine was administered directly to those sites. Similar results were obtained in human studies (23). After uptake by the rat jejunum, some of the absorbed pyridoxine is phosphorylated in the intestinal mucosa (24, 25), which then may be dephosphorylated by a phosphatase enzyme (25). The rates of uptake and phosphorylation are not altered in vitamin B-6 deficient rats (26).

Studies of the absorption of tritium-labelled pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP)



Vitamin B-6

<u>R</u>	<u>Nomenclature</u>
CH ₂ OH	Pyridoxine
CHO	Pyridoxal
CH ₂ NH ₂	Pyridoxamine

Phosphate derivative
of Vitamin B-6

<u>R</u>	<u>Nomenclature</u>
CH ₂ OH	Pyridoxine 5'-Phosphate
CHO	Pyridoxal 5'-Phosphate
CH ₂ NH ₂	Pyridoxamine 5'-Phosphate

Figure 1. Chemical structures and nomenclature of vitamin B-6 vitamers.

revealed that under normal physiological conditions the majority was dephosphorylated to pyridoxal and pyridoxamine, respectively, in the intestinal lumen, and then absorbed by passive diffusion (27-29). However, direct absorption of PLP and PMP, albeit at a slower rate, was also demonstrated. In experiments with everted sacs of rat small intestine, comparison of the absorption rates of the three forms of vitamin B-6 revealed quantitative differences (30, 31). The rate of transport across the intestinal wall decreased in the following order: pyridoxal > pyridoxamine > pyridoxine, while the rate of uptake in the jejunal tissue decreased in the order: pyridoxal > pyridoxine > pyridoxamine which paralleled the order of phosphorylation of these compounds.

In contrast to the intestinal absorption of vitamin B-6 by passive diffusion, the transport of vitamin B-6 across the human placenta appears to involve an active mechanism. This is evidenced by the large positive gradient of total vitamin B-6 and individual vitamers in the cord blood over that of the maternal blood (32-34).

The bioavailability and stability of vitamin B-6 in foodstuffs are important factors in estimating the dietary intake of the vitamin. The bioavailability and stability of vitamin B-6 depend upon such factors as food processing, storage, and diet composition. Pyridoxamine and pyridoxal predominate in animal tissues while pyridoxine is found in higher concentrations in plant materials (35). The effect of

thermal processing on vitamin B-6 in milk products was first observed in the early 1950's when infants fed a commercially sterilized formula developed convulsions which were responsive to treatment with vitamin B-6 (36). The same infant formula, spray-dried rather than sterilized, did not cause convulsions. Also, rat bioassay techniques indicated a lower vitamin B-6 content of heat-sterilized milk products than microbiological assay methods while both techniques agreed when measuring the vitamin B-6 content of spray-dried milk products (37). Although the mechanism is not well understood, these results indicate that thermal processing may significantly affect the bioavailability of vitamin B-6 in foods.

The stability of vitamin B-6 in foods is also affected by thermal processing. Roasting of various meats resulted in a 50% loss of total vitamin B-6 content (38), and canning of several varieties of beans resulted in an approximate 20% loss (39, 40). Roasting of dehydrated model food systems (simulating breakfast cereal) was found to degrade 50 - 70% of the pyridoxine, pyridoxamine, and PLP (41). Subsequent storage of the dehydrated food systems resulted in vitamin B-6 degradation which followed first order kinetics (42).

Storage temperature and the pH of the food have been shown to affect the stability of vitamin B-6 during storage. Storage of canned U.S. Army rations for 20 months resulted in a 30% loss of vitamin B-6 at 100°F compared with storage at 34°F (43). Heat-treated and irradiated foods stored at

room temperature showed a 40% - 60% loss of vitamin B-6 activity compared with frozen storage (44).

While loss of vitamin B-6 occurred during retort processing and storage in low-acid foods such as lima beans and beef, no such loss took place in the canned tomato juice concentrate, a high-acid product (39). Little loss of vitamin B-6 has been observed during storage of vitamin B-6 fortified flour and bread. The stability of pyridoxine-HCl, the form used to fortify foods, has been reported to be very good (45, 46). However, the bioavailability of this vitamin in a fortified rice breakfast cereal has been found to be low (47).

Tarr et al. (48) estimated the bioavailability of an "average" mixed American diet to be 71% - 79% when compared with semi-purified formula diet providing pyridoxine-HCl as the sole source of vitamin B-6. While vitamin B-6 has been reported to be fully available in dried beef and lima beans, the vitamin B-6 in whole wheat flour and non-fat dry milk is only about 80% available (49). The bioavailability of vitamin B-6 from whole-wheat bread was 5 - 10% less than from bread fortified with pyridoxine-HCl and from white bread plus a pyridoxine-HCl oral supplement (50).

Intraluminal perfusions of human jejunum revealed that absorption of vitamin B-6 from orange juice was less than from synthetic saline and glucose solutions containing vitamin B-6 (51) which may be due to binding of the vitamin B-6 in orange juice to a low molecular compound (52).

Recently, the presence of a natural vitamin B-6 derivative, pyridoxine 5'- β -glucoside has been reported in some cereals and seeds (53, 54). Among other factors which may influence vitamin B-6 bioavailability in foods are the presence of vitamin B-6 antimetabolites or degradation products such as ϵ -pyridoxyllysine, which may have anti-vitamin B-6 activity under certain conditions (55-57).

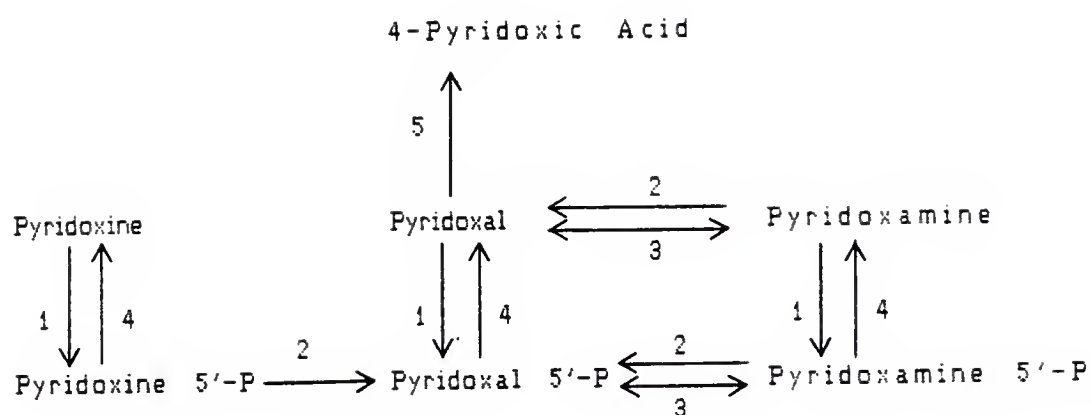
Vitamin B-6 Metabolism

As a cofactor for more than 60 enzyme systems, PLP is the main metabolically active form of vitamin B-6 although PMP occasionally also serves as a coenzyme. PLP-dependent reactions in amino acid metabolism include decarboxylation, transamination, deamination, racemization and desulphydration (58).

Of particular interest in connection with vitamin B-6 is the metabolism of tryptophan, tyrosine, methionine, cysteine, glutamate, serine, glycine, aspartate and alanine (59-64). PLP is required for numerous synthetic and degradation reactions of compounds functioning as neurotransmitters in the central nervous system including dopamine, norepinephrine, epinephrine, serotonin, gamma aminobutyric acid (GABA), taurine and histamine (62, 65, 66). Lipid and carbohydrate metabolic reactions also involve vitamin B-6 (67-69). PLP plays an important structural role in the enzyme phosphorylase which catalyzes the breakdown of glycogen to glucose-1-phosphate (67). The formation of

δ -aminolevulinic acid, an intermediate in the synthesis of heme, requires PLP (70). Vitamin B-6 is involved in the synthesis of other vitamins and coenzymes. In its very active role in the tryptophan metabolic pathway, PLP is also essential to the synthesis of niacin (71). It is also required for the production of N⁵,N¹⁰ methylene tetrahydrofolate which is necessary for the synthesis of deoxythymidylate and purines (72). The formation of coenzyme A from pantothenate also involves PLP (72). Vitamin B-6 also plays a role in endocrine metabolism and is required in the synthesis of a variety of hormones (72).

The metabolic interconversions of the vitamin B-6 vitamers are illustrated in figure 2. PLP is synthesized by phosphorylation of pyridoxal by pyridoxal kinase or phosphorylation of pyridoxine and pyridoxamine followed by oxidation by pyridoxamine (pyridoxine) 5'-phosphate oxidase. Dephosphorylation of PLP, PMP and PNP is accomplished by various phosphatases. Pyridoxal and pyridoxamine and PLP and PMP can also be interconverted via transamination (73). Transamination reactions which require PLP as a coenzyme involve a Schiff base mechanism whereby PLP and PMP are interconverted (71). Pyridoxal is oxidized to the inactive metabolite 4-pyridoxic acid by either aldehyde oxidase or an unspecific NAD⁺-linked aldehyde dehydrogenase (74). PLP and PMP are the predominant B-6 vitamers in mammalian tissues (75, 76), and the PMP:PLP ratio appears to vary with the type of tissue and vitamin B-6 status.



- 1 Pyridoxal kinase
- 2 Pyridoxamine (pyridoxine) 5'-phosphate oxidase
- 3 Aminotransferases
- 4 Alkaline phosphatases
- 5 Aldehyde oxidase and dehydrogenase

Figure 2. General pathway of mammalian vitamin B-6 metabolism.

Glycogen phosphorylase represents the major vitamin B-6 reservoir in the body since about 90% of the vitamin B-6 in muscle is associated with this enzyme, up to 5% of muscle protein is glycogen phosphorylase and 40% of the body is muscle (77-79). Pyridoxal and PLP in the circulation are the major transport forms of vitamin B-6 in the body. PLP represents more than 50% of the plasma B-6 vitamers under normal conditions (80), and most of the PLP is bound to albumin (81, 82). Plasma PLP clearance rate in man ingesting a normal diet has been reported to be 31.7 ± 2.7 ml/min (83), which indicates a rapid turnover rate. The turnover rate of PLP in erythrocytes is much slower (84), and therefore it represents a different metabolic pool of PLP in the body. PLP appears to be about equally distributed between erythrocytes and plasma in the blood of normal subjects on regular diets (84). PLP has been shown to interact with hemoglobin (85) and may therefore be bound principally to hemoglobin in red blood cells (86). Although free PLP is able to enter red blood cells, PLP complexed with albumin does not (81, 87, 88). Racial differences in the activity of pyridoxal kinase in erythrocytes have been reported (89, 90). However, pyridoxal kinase activity does not appear to be an important regulator of red cell PLP levels under normal dietary conditions although it does play a role when pharmacological doses are administered (91).

The liver appears to be a crucial tissue in the metabolism of vitamin B-6 and has been shown to be the sole

source of plasma PLP (81). The unphosphorylated B-6 vitamers absorbed by the intestine are rapidly metabolized in the liver to PLP, most of which are bound to various intracellular proteins including glycogen phosphorylase and cytosolic alanine and aspartate aminotransferases (92). Pyridoxamine (pyridoxine) phosphate oxidase activity, which is regulated in vivo by product inhibition, is one factor in the control of synthesis of liver PLP (93). Under normal conditions most of the newly synthesized PLP is released into the circulation, and the remainder is metabolized to pyridoxal and 4-pyridoxic acid and also secreted. The major regulation of PLP concentration in the liver is controlled by a balance between hydrolysis of PLP by phosphatase and the protection of PLP against hydrolysis by protein-binding (94).

This type of regulation has also been demonstrated to exist in other body tissues including erythrocytes (87) and the brain (95). There is evidence that vitamin B-6 enters the central nervous system from the plasma by a saturable transport mechanism (96) and that the choroid plexus serves as the primary site of entry and is the main source of phosphorylated vitamin B-6 in the cerebrospinal fluid (97).

The total body store of vitamin B-6 has been reported to range from 40 to 150 mg pyridoxine equivalents as estimated by administration of tritium-labeled pyridoxine to human subjects (98). In this study 2% to 3% of the vitamin B-6 reservoir was eliminated per day, and the total turnover

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Abstract of Dissertation Presented to the Graduate School
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DETERMINATION OF THE VITAMIN B-6 REQUIREMENT
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By

Karen Schuster

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Chairman: Dr. Lynn B. Bailey
Major Department: Food Science and Human Nutrition

The objective of this study was to determine the vitamin B-6 requirement of pregnant women by assessing the effect of graded doses of maternal pyridoxine supplementation on the biochemical indicators of vitamin B-6 status and on the condition of their infants at birth. The 196 volunteer subjects were low-income patients attending Maternal and Infant Care (MIC) clinics in north central Florida and ranged in age from 17 to 38 years. The mean stage of pregnancy at the initial clinic visit was 15 ± 4 weeks and ranged from 6 to 21 weeks. At the first prenatal clinic visit subjects were randomly assigned a daily vitamin B-6 supplement containing 0, 2.6, 5, 7.5, 10, 12.5, 15 or 20 mg of pyridoxine-HCl. Maternal health and vitamin B-6 status of the subjects were assessed at three stages of pregnancy:

first prenatal clinic visit, 30 weeks gestation, and at term. Vitamin B-6 status and condition of the infants at birth were also determined.

The mean dietary vitamin B-6 intake of the group was 1.43 ± 1.28 mg/day as estimated from 24-hour dietary recalls. Maternal plasma PLP levels were positively correlated with vitamin B-6 supplementation at 30 weeks gestation ($r=0.55$, $p<0.0005$) and at delivery ($r=0.54$, $p<0.01$). However, cord plasma PLP levels did not increase linearly with the level of vitamin B-6 supplementation but reached a maximum at 7.5 mg and greater. Supplemental pyridoxine-HCl at the 5 mg level was required to maintain plasma PLP at 30 weeks gestation at a level comparable to initial values; however, 7.5 mg were required to prevent a decrease in maternal plasma PLP at delivery from the initial level. Apgar scores at 1 minute after birth were significantly higher ($p<0.05$) for infants whose mothers took 7.5 mg or more supplemental pyridoxine-HCl than for infants of mothers who took 5 mg or less. These findings indicate that the approximate vitamin B-6 requirement of pregnant women is between 5.5 and 7.6 mg/day (diet plus supplement as pyridoxine equivalents) to ensure adequate vitamin B-6 status and optimal health of the infant at birth.

rate was 2.2% to 4.4% per day. 4-Pyridoxic acid was the major urinary vitamin B-6 metabolite excreted, accounting for 20% to 40% of the isotope eliminated.

In summary, pyridoxal is the form that is transported across cell membranes into extrahepatic tissues where it is rephosphorylated and bound to intracellular proteins and enzymes. Plasma PLP bound to albumin represents a circulating storage pool since protein-binding effectively protects it from hydrolysis and the binding capacity with albumin is large. Metabolism of vitamin B-6 in various tissues has recently been reviewed (86, 99).

Vitamin B-6 Requirements

The 1980 Recommended Dietary Allowances (RDA) for vitamin B-6 by the Food and Nutrition Board of the National Research Council are 2.2 mg/day for men 19 years and older, 2 mg/day for females 15 years and older, 2.6 mg/day for pregnant women, 2.5 mg/day for lactating women and 0.3 mg/day for infants up to 6 months of age (100). These RDAs are based on limited information regarding the vitamin B-6 requirements of these groups.

The requirement for vitamin B-6 of humans has been difficult to establish since clinical manifestations of mild vitamin B-6 deficiency are nonspecific and not readily recognizable. Primarily, the requirement for vitamin B-6 has been estimated by the amount of the vitamin required to

normalize the urinary excretion of tryptophan and methionine metabolites after tryptophan and methionine loading (100, 101). Since vitamin B-6 functions as a coenzyme in the metabolism of amino acids, disturbances in amino acid metabolism have proved to be sensitive indicators of vitamin B-6 deficiency (102).

Using the method of vitamin B-6 depletion and repletion with various biochemical measurements, a series of studies involving small groups of young adult men were conducted by two research groups: the U.S. Army Medical Research and Nutrition Laboratory and the University of Wisconsin. The U.S. Army research group gave a synthetic vitamin B-6 deficient diet containing 0.06 mg of vitamin B-6 to eight subjects (103) while the Wisconsin group provided a partially purified diet with 0.16 mg of vitamin B-6 to six subjects (104, 105, 106, 107). A tryptophan load of 10 mg of DL-tryptophan (U.S. Army) or 2 mg L-tryptophan (Wisconsin) was administered to challenge the tryptophan metabolic enzyme systems. Although other biochemical measurements were determined including blood and urinary concentrations of various vitamin B-6 vitamers, the vitamin B-6 requirement was determined from the tryptophan load test since the other measurements proved to be insufficient to assess the requirement. In general, blood and urine levels of vitamin B-6 vitamers and 4-pyridoxic acid decreased during vitamin B-6 depletion and increased with repletion. The effect of

the level of dietary protein on the vitamin B-6 requirement was also investigated in these experiments.

In the U.S. Army study, half of the subjects were fed a low protein diet (30 g/day) while the rest were given a high-protein diet (100 g/day) (103). After three weeks on the vitamin B-6 deficient diet (0.06 mg/day), the tryptophan load test was administered, and xanthurenic acid excretion was measured. Pyridoxine supplementation then was initiated, and pyridoxine levels were titrated with weekly urinary xanthurenic acid measurements until the minimal vitamin B-6 requirement was met. The pyridoxine requirement was estimated to be 1.5 mg/day for the high-protein group and 1.0 mg/day for the low-protein group.

In one experiment, the Wisconsin group found that 0.6 or 0.9 mg/day of supplemental pyridoxine was insufficient to restore tryptophan metabolite excretion to predepletion levels in all subjects (104). In a second study, five subjects were given 54 g/day dietary protein, and six were fed 150 g/day (107). The high-protein group developed abnormal metabolism of tryptophan in about one-third the time that the low-protein group did. In both groups before depletion, 1.5 mg of pyridoxine were sufficient to maintain normal excretion levels of tryptophan metabolites; but after depletion, 1.06 mg of pyridoxine were insufficient to restore excretion levels to normal. When a similar study was conducted in which the effect of vitamin B-6 deficiency on methionine metabolism was investigated, 2.16 mg of

pyridoxine given daily during the repletion period were barely adequate to normalize methionine metabolism in subjects consuming 150 g/day of protein.

Taken together, these data indicate that the vitamin B-6 requirement is between 1.5 and 2.0 mg/day for men consuming 100 - 150 g/day of protein and between 1.0 and 1.5 mg/day for men with a dietary protein intake less than 100 g/day. The requirement appears to be greater than 2.0 mg/day when dietary protein intake exceeds 150 g/day.

The vitamin B-6 requirement of young women was determined by two research groups, one at Cornell University using 8 subjects (108) and the other at the University of Wisconsin with 5 subjects in one experiment (109) and with 10 subjects in a later experiment (110, 111). There were differences in the experimental methods among the three studies including dietary protein levels; length of pre-depletion, depletion and repletion periods; and levels of dietary vitamin B-6 and supplemental pyridoxine. Despite these differences, similar conclusions were reached. The requirement of young women for vitamin B-6 appears to be between 1.5 and 2.2 mg/day based on the amount of pyridoxine required to return various biochemical parameters to pre-depletion levels. Two of the research groups reported that although aspartate aminotransferase activity in erythrocytes decreased during vitamin B-6 depletion, supplementation with pyridoxine up to 2.2 mg/day failed to restore activity to original levels (108, 110). The

Wisconsin group observed that in vitro stimulation of the enzyme system by addition of PLP resulted in an increase of enzyme activity during depletion and a decrease with repletion although original levels were not reached (110). The Cornell group found that alanine aminotransferase activity failed to respond to either vitamin B-6 depletion or repletion (108) while the Wisconsin group reported a decrease in activity with depletion and an increase (without attaining predepletion levels) during repletion (110). Stimulation of the enzyme with PLP was variable and inconclusive. Urinary excretion of 4-pyridoxic acid was measured in both studies, and 1.0 mg/day of supplemental pyridoxine was insufficient to restore original levels, 1.5 mg/day was borderline and 2.2 mg/day was excessive (108, 110). Either a 1.0 or 2.2 mg/day intake of pyridoxine was sufficient to restore urinary tryptophan metabolites to predepletion levels (109, 111). Results from methionine loading tests were inconclusive due to variable data (109). Plasma PLP levels exceeded predepletion levels with 2.0 mg/day of pyridoxine while 0.8 mg only brought plasma PLP to 50% of the original values (111).

These data form the basis of the RDA established for adult men and women in the United States. The allowance for vitamin B-6 set by the Canadian Bureau of Nutritional Sciences is also based on these data but evaluated in an alternate way. The Dietary Standard for Canada recommends an intake of 0.02 mg of vitamin B-6 per gram of dietary protein

(112). From the data in the studies reporting both vitamin B-6 and protein intake, it was calculated that a vitamin B-6/protein ratio of 0.017 was inadequate while 0.019 was sufficient to return the majority of biochemical indices to predepletion values.

The RDA for vitamin B-6 for pregnant women is extrapolated from the vitamin B-6 requirement of nonpregnant women. The increase in the RDA during pregnancy from 2 to 2.6 mg is based on the additional vitamin B-6 needed for the increased protein allowance in pregnancy (100). No additional vitamin B-6 is recommended to compensate for fetal demand, increased maternal metabolic requirements and hormonal induction of maternal vitamin B-6 dependent enzymes found to occur during pregnancy (32, 33, 113-115).

Numerous studies have demonstrated that a significant number of pregnant women who consume a normal diet or receive vitamin B-6 supplements containing the RDA have low levels of various biochemical parameters consistent with a vitamin B-6 deficiency in non-pregnant women (32, 33, 113-114, 116, 117). However, the Committee on Dietary Allowances of the Food and Nutrition Board has indicated that although the establishment of the vitamin B-6 RDA during pregnancy is "beset with uncertainties," there are insufficient data to justify recommending a higher allowance which would exceed that provided by the usual diet (100).

The vitamin B-6 RDAs for infants and lactating women are also established from very limited data. The RDA for

infants is based on the vitamin B-6 content of breast milk and additional infant foods and the protein intake of the infants (118). The additional 0.5 mg in the recommended allowance during lactation above the nonpregnant RDA is based on information that women consuming vitamin B-6 at that level seem to provide the needs of the breast-fed infant (100).

Vitamin B-6 Deficiency

Impairment of growth, the appearance of a pellagra-like dermatitis (acrodynia), and the development of ataxia are commonly observed effects of a vitamin B-6 deficiency in various animal species (70). A hypochromic microcytic anemia which is responsive to vitamin B-6 administration occurs in many species (119). Vitamin B-6 deficiency has a profound effect on the nervous system. In addition to ataxia, these nervous system abnormalities have been observed in various species: hyperacousis, hyperirritability, altered mobility and alertness, abnormal head movement and convulsions (65). Vitamin B-6 deficiency in the rat also results in muscular weakness, fatty liver, convulsive seizures, reproductive impairment, edema, enlarged adrenal glands, and impaired immune responses (70). Alterations in various biochemical processes have been observed, including increased excretion of xanthurenic acid, urea and oxalate, decreased transaminase activities, reduced synthesis of ribosomal and

messenger ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) (70).

Clinical manifestations of vitamin B-6 deficiency in humans are less obvious. A vitamin B-6 deficient diet alone produces only nonspecific symptoms of deficiency such as mental depression, irritability and oral lesions. Vitamin B-6 deficiency has been induced experimentally by the use of vitamin B-6 antagonists such as 4-deoxypyridoxine. In such studies, the following symptoms developed in some of the subjects: oral lesions, dermatitis, peripheral neuropathy, weight loss, apathy, insomnia, irritability, slight decrease in immune response, and abnormal electroencephalograms (120-122).

Dramatic manifestations of a vitamin B-6 deficiency occurred in human infants fed a proprietary liquid canned milk formula later found to be vitamin B-6 deficient due to thermal processing. These symptoms included irritability, nervousness, convulsive seizures, and abnormal electroencephalograms which were promptly reversed by intramuscular injections of 100 mg of pyridoxine (36, 123). These convulsions are thought to be related to decreased levels of γ -aminobutyric acid, an inhibitory neurotransmitter formed from glutamic acid in a vitamin B-6 dependent reaction (124). A report regarding two infants after several months on a vitamin B-6 deficient diet indicated that convulsions occurred in one and hypochromic anemia in the other (125). Both ceased to gain weight.

Urinary excretion of 4-pyridoxic acid ceased while excretion of vitamin B-6 was reduced to very low levels. Also, the ability to convert tryptophan to nicotinic acid was impaired.

Numerous changes in biochemical processes have been reported to occur in vitamin B-6 deficient humans. Tryptophan metabolism is altered resulting in high urinary excretion of xanthurenic acid and other metabolites after a tryptophan challenge (102). This pathway, which requires PLP as a coenzyme at various points, is illustrated in figure 3. High dietary intake of protein hastens the onset of vitamin B-6 deficiency (126). Plasma and blood levels of vitamin B-6 as well as urinary excretion of vitamin B-6 and 4-pyridoxic acid are decreased in vitamin B-6 deficiency (102). Alterations in methionine metabolism have been reported (127). Incorporation of cystine into hair, which consists of protein with a high cystine content, is reduced in vitamin B-6 deficiency which may explain the partial acrodynia observed in animals (128). Decreased erythrocyte alanine and aspartate aminotransferase activities and coenzyme saturation of these enzymes have been observed in vitamin B-6 deficiency (102). Changes in plasma and urinary levels of amino acids have also been reported (126).

A number of conditions and diseases may lead to vitamin B-6 deficiency in humans. Low plasma PLP levels and decreased activities of aminotransferases have been reported in alcoholics and patients with liver disease and uremia

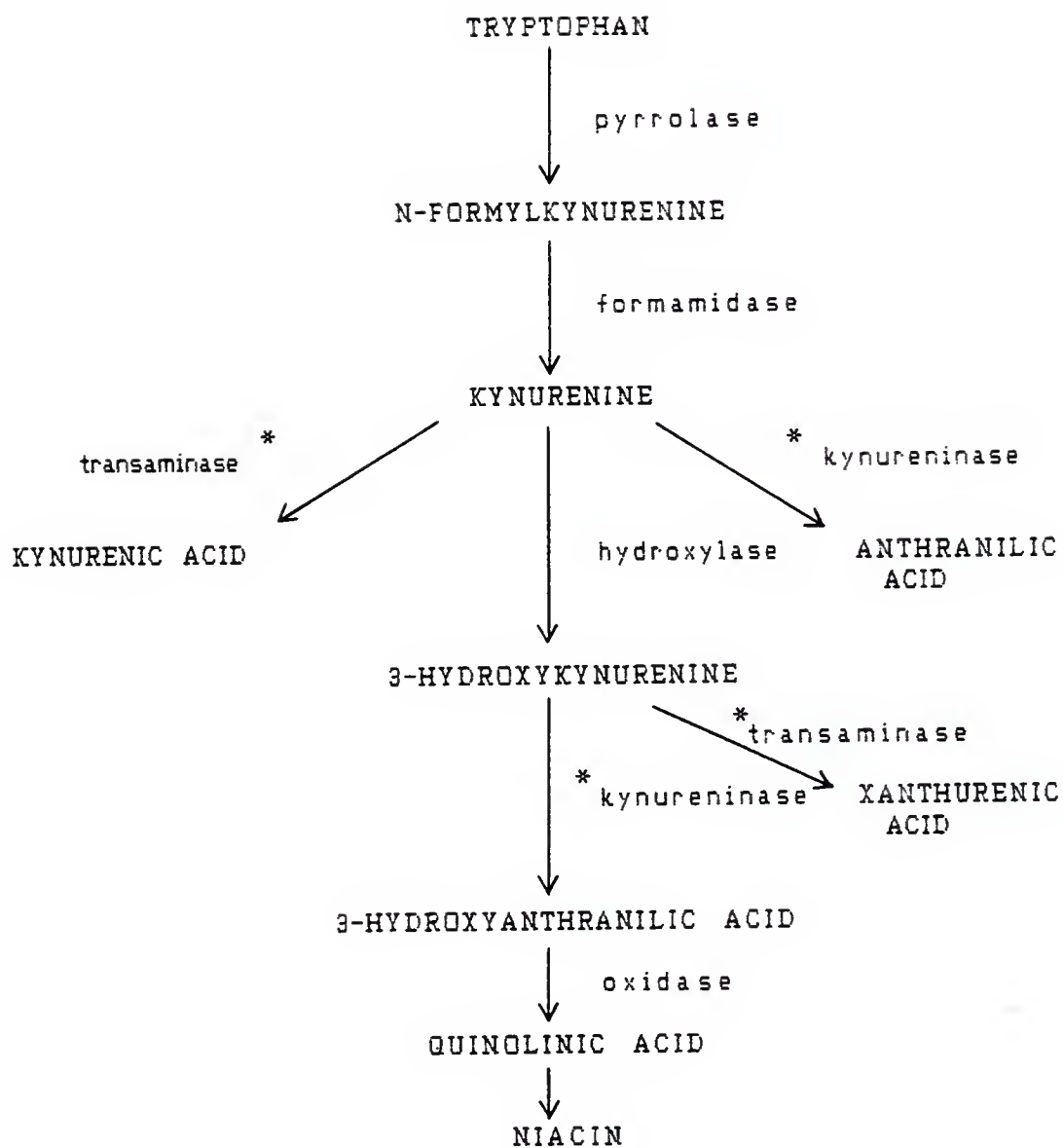


Figure 3. Tryptophan to niacin pathway in the human. Asterisks indicate reactions which require PLP as a coenzyme.

(83, 129, 130). The decreased PLP levels are thought to result from increased clearance of the coenzyme from the circulation (130). There is evidence of increased PLP degradation by the diseased liver (129). Recently, hydrolysis of PLP in plasma was demonstrated in patients with liver disease and other conditions with raised alkaline phosphatase, whether of liver or bone origin (131).

Abnormal responses to tryptophan load tests suggestive of a vitamin B-6 deficiency have been reported in patients with a variety of diseases, including Hodgkins' disease, rheumatoid arthritis, schizophrenia, porphyria, tuberculosis, aplastic anemia, scleroderma, and various cancers (132). It has been suggested that liver tryptophan oxygenase is induced by the increased adrenal secretion of cortisol brought about by the stress of these diseases (133).

Abnormal tryptophan metabolism as well as low plasma PLP and erythrocyte aminotransferase activities have also been observed in pregnant women and oral contraceptive users (134-136). It has been demonstrated that the abnormal tryptophan metabolism results from the induction of tryptophan oxygenase by estrogen, either present in oral contraceptives or from endogenous secretion in pregnancy (134, 135). The effects of estrogen on tryptophan metabolism have been extensively studied and involve both direct effects on tryptophan metabolism as well as cortisol-mediated indirect effects (132).

A number of clinical situations exist in which the requirement for vitamin B-6 is unusually high. Some patients with the inherited metabolic diseases homocystinuria and cystathioninuria respond to pharmacological doses of pyridoxine (137, 138). Patients with a vitamin B-6 responsive sideroblastic anemia require the administration of 2.5 g or more of pyridoxine daily (70). Certain infants with convulsive seizures show a high requirement for vitamin B-6, and dosages between 2 - 5 mg have relieved convulsive activity (139).

The effects of vitamin B-6 deficiency on the fetus during gestation have been extensively studied in animals. The rat model has been used particularly for experimentation involving postnatal insults on brain development since the most rapid increase in the growth rate of the rat brain occurs approximately 10 days after birth (140). In the human the most rapid increase in brain growth rate takes place just before birth.

Early rat studies demonstrated that the progeny of severely vitamin B-6 deficient pregnant rats exhibited convulsive seizures, lower birth weights, and lower brain PLP levels than controls (141). Body weights of litters of rat dams which received 0, 25 or 50% of the NRC recommendation for vitamin B-6 were significantly lower than those of dams receiving 75, 100 or 400% of the NRC recommended intake (142). Also, neuromotor activity was delayed in the vitamin B-6 restricted groups. The onset of

crawling was delayed one day for the 25% group versus the 75% group, and standing was delayed four days in the 25% group compared with the 100% group.

Further studies confirmed these results and indicated that the impaired neuromotor development of the pups was due in part to the impaired maternal performance of the dams due to vitamin B-6 deficiency (143). Pups whose mothers had received graded levels of vitamin B-6 (0, 25, 50, 75, 100, or 400% of the NRC recommended intake) were cross-fostered with a dam isonutritional with the pup's mother or with a control dam receiving a diet containing 400% of the NRC recommendation for vitamin B-6. The data indicated that vitamin B-6 deprivation during gestation operated in two ways: directly, by impairing the development of the fetus; and indirectly, by adversely affecting the ability of the dam to function as a mother which was not easily reversed by an adequate post-parturition diet.

A similar experiment indicated that the effects of vitamin B-6 deprivation during lactation generally were not as severe as those seen during gestation (144). Rats which received 400% of the NRC recommended intake for vitamin B-6 during gestation were fed 0, 25, 50, 75, 100, or 400% of the NRC recommendation during lactation. Pups suckling dams which received 100% or less of the NRC recommended intake during lactation were later in the onset of the more advanced neuromotor skills than those whose mothers received the 400% diet. These data indicated that the prenatal

vitamin B-6 requirement of rats is greater than the postnatal requirement and that the current NRC recommended intake of vitamin B-6 for rats may be insufficient for the optimum growth and development of the progeny.

A 30 to 50% reduction in cerebral sphingolipid level has been reported in vitamin B-6 deficient suckling rats as well as severe deviation of cerebral free amino acids from the normal (145). Sphingolipids are essential for the proper development of the nervous system, particularly for myelination. It has been postulated that sphingosine synthesis is reduced in vitamin B-6 deficiency due to decreased activity of the vitamin B-6 dependent synthase enzyme which forms 3-dehydrosphinganine from palmitoyl CoA and serine.

Vitamin B-6 deficient suckling rats exhibited reduced recoverable myelin in the brain which was not attributable to decreased brain weight alone (146). Myelin recovery, total myelin lipids, and cerebral vitamin B-6 content were inversely related to the duration of the vitamin B-6 deprivation. The polyunsaturated fatty acid content of the myelin phospholipids was markedly reduced in severely vitamin B-6 deficient pups. However, the relationship between the synthesis of long-chain polyunsaturated fatty acids and vitamin B-6 is not known. The activity of 3-dehydrosphinganine synthase in the vitamin B-6 deficient pups was only 62% of the control group. However, enzyme activity increased to normal levels when PLP was added in

vitro, demonstrating that the reduced enzyme activity was due to vitamin B-6 deficiency and not to lack of apoenzyme.

Twelve-day old rat pups whose dams were fed low levels of vitamin B-6 throughout growth, gestation and lactation exhibited reduced brain levels of vitamin B-6 and cerebrosides (147). Total lipid, phospholipid, cholesterol and proteolipid content were similar in all treatment groups. Brain lipid profiles of progeny of vitamin B-6 deficient rats before and after vitamin B-6 supplementation were studied (148). Cerebroside and ganglioside content were significantly reduced in pups from unsupplemented vitamin B-6 deficient dams and from dams supplemented beginning ten days postpartum. Supplementation of vitamin B-6 deficient dams five days postpartum normalized levels of these brain lipids in the offspring.

Cytoarchitectural alterations in the brains of rat pups of vitamin B-6 deficient dams have been observed, including reduced neocortex and cerebellum areas and adversely affected Purkinje cell differentiation (149). Although maternal vitamin B-6 deficiency during the period of rapid myelination (10 to 20 days postnatal in the rat) did not result in decreased myelination in the brains of progeny (150), decreased myelination in the spinal cords of progeny of vitamin B-6 deficient dams has been observed (151).

Administration of the vitamin B-6 antagonist 4-deoxypyridoxine to rat dams deprived of vitamin B-6 during gestation inhibited replication and function of lymphocytes

involved in cell-mediated immunity of the progeny (152). Passive antibody transfer during lactation from dams to rat pups as measured by immunoglobulin G concentrations in maternal serum, pup serum, and milk was not affected by maternal vitamin B-6 deficiency (153). However, suckling rats of vitamin B-6 deficient dams and weanling rats consuming a vitamin B-6 deficient diet had fewer spleen cells, splenic antibody-forming cells and lower levels of circulating antibodies than non-deficient groups. This reduction resulted partly from lower food intake but was intensified by vitamin B-6 deprivation.

Methods of Vitamin B-6 Status Assessment and Vitamin B-6 Analysis

Methods to assess vitamin B-6 status rely mainly upon dietary intake and biochemical data since clinical signs of vitamin B-6 deficiency are nonspecific and are rarely seen in a free-living population. Biochemical methods which have been used to evaluate vitamin B-6 status include blood and urinary levels of the vitamin B-6 vitamers or metabolites, activities of vitamin B-6 dependent enzymes in blood, and levels of urinary metabolic products (102).

Estimation of dietary intake of nutrients, including vitamin B-6, has been limited for practical reasons to two methods: 24-hour dietary recalls or 3-day diet records. The 24-dietary recall method has been criticized for a variety of reasons (154, 155). However, when the technical limitations are recognized in interpreting data from

24-dietary recalls, it is generally agreed that 24-hour recalls provide estimates of group intakes that are comparable to results obtained with more cumbersome techniques (156). It has been pointed out that while the mean and median intake values for the group obtained by 24-hour dietary recall method have validity, it is incorrect to assign percentile positions or ranks to individuals in the group and then generalize about the individual (157). A statistical examination of sources of variance in 24-hour dietary recall data has been performed (156). Potential sources of error in the recall method include the recall ability of the subject and the accuracy and completeness of food composition data.

Most reports which have included vitamin B-6 dietary intake information have used the 24-dietary recall (117, 136, 158). Roepke and Kirksey (159) compared the results obtained from 24-dietary recalls and 3-day diet records and found a correlation of 0.78 for the vitamin B-6 intake estimated by the two methods. The 3-day diet record provided a slightly larger estimate of intake than the 24-dietary recall.

Vitamin B-6 status has been assessed with direct methods by measuring vitamin B-6 vitamers in blood, urine and other tissues. Indirect methods which have been used include measurement of tryptophan or methionine metabolite excretion following a loading dose of the amino acid and determination of activities of vitamin B-6 dependent enzymes

before and after adding PLP. The tryptophan load test has been more frequently used than the methionine loading procedure. After a 2 or 5 gram oral loading dose of L-tryptophan, urinary xanthurenic acid, 3-hydroxykynurenine, and kynurenine are measured by spectrophotometric, colorimetric or fluorometric methods following separation by column chromatography (111). Although this method provides a measure of the functional adequacy of coenzyme levels, many factors other than vitamin B-6 status can influence urinary excretion of these metabolites including protein intake, lean body mass, exercise, individual variations, the size of the amino acid loading dose, and estrogen levels (102). Another disadvantage is that this procedure requires a 24-hour urine collection which is often not practical. Consequently, this method is not used currently although many of the vitamin B-6 requirement studies utilized this procedure (33). Because of the marked derangement of tryptophan metabolism caused by estrogen through the induction of tryptophan oxygenase, the tryptophan load test is not useful in assessing vitamin B-6 status in pregnant women or oral contraceptive users.

Transaminase activities in blood also provide a functional measurement of the state of vitamin B-6 reserves. Transaminase activities have been measured in erythrocytes, leukocytes and plasma. Since plasma transaminase activities are much lower than in erythrocytes and show a wide range in normal individuals, they are not considered useful in

assessing vitamin B-6 status. The two transaminases measured in erythrocytes are alanine aminotransferase (AlaAT, E.C. 2.6.1.2) and aspartate aminotransferase, (AspAT, E.C. 2.6.1.1), also referred to as glutamic pyruvate transaminase (GPT) and glutamic oxaloacetate transaminase (GOT), respectively.

Several reports have indicated that measurement of these enzyme activities per se did not give consistent results but that the degree of stimulation by PLP added in vitro was a better indicator of vitamin B-6 status (160-162). More recently, Lumeng et al. (163) demonstrated that in general erythrocyte AspAT and AlaAT activities were more sensitive indicators of vitamin B-6 status than stimulation values. AspAT activity has been reported to be higher than AlaAT in all tissues measured in the rat and is therefore more easily determined (164). There are conflicting reports as to which enzyme provides the better measurement. Cinnamon and Beaton (160) found that erythrocyte AlaAT stimulation by PLP was more sensitive to dietary vitamin B-6 depletion and repletion than stimulation of AspAT. However, Donald et al. (108) found the converse to be true. In a recent report of the vitamin B-6 requirement of oral contraceptive users assessed by erythrocyte AspAT and AlaAT activities and stimulation with PLP, Bosse and Donald (165) observed random fluctuations in AspAT activity during vitamin B-6 depletion and repletion. Although erythrocyte AlaAT activity was sensitive to vitamin B-6

deficiency, it was slow to respond to pyridoxine supplementation.

A number of factors may contribute to the decreased sensitivity of the aminotransferase activity measurements and stimulation values, including variations in the assay procedures used (166). Normal healthy individuals appear to have a wide range of activity and stimulation values (102). Stimulation of aminotransferase activities may be substrate concentration dependent (167). Sample handling may be a factor since intracellular changes in the degree of saturation of the apoenzyme after cell death have been observed (168). Since PLP is tightly bound to both enzymes, saturation decreases only in relatively severe vitamin B-6 deficiency. Transfer of PLP to AspAT and AlaAT from low-affinity binding proteins such as the vitamin B-6 dependent enzymes serine dehydratase and tyrosine aminotransferase has been shown to occur in rat liver (163). Some aminotransferases appear to be induced by hormones (169).

Consequently, erythrocyte AlaAT and AspAT activity and stimulation measurements have not been significantly correlated with other parameters of vitamin B-6 status such as plasma PLP levels in numerous studies, particularly in pregnant subjects (113, 117, 170, 171). Reasonable correlations have been obtained when vitamin B-6 deficiency is relatively severe or pyridoxine supplementation is given (113, 163).

The AspAT and AlaAT activities have been assayed colorimetrically (166, 172), but these procedures have been criticized for their lack of specificity (173, 174). The recommended method for measuring serum AspAT and AlaAT activities is a coupled enzyme spectrophotometric procedure which has been adapted to erythrocytes (175). In this method the oxaloacetate or pyruvate formed from the transaminase reaction is reduced by NADH_2 in a malic or lactic dehydrogenase catalyzed reaction, and the reaction rate is monitored by a decrease in absorbance at 340nm as NADH_2 is oxidized. Recently, this procedure for measuring AspAT and AlaAT activities has been optimized (176) and adapted for use in automated systems (177).

Measurements of the major urinary excretory metabolite of vitamin B-6, 4-pyridoxic acid, and to a lesser extent, free vitamin B-6, have been used to assess vitamin B-6 status. It has been assumed that excretion of 4-pyridoxic acid reflects vitamin B-6 intake since urinary levels decrease during vitamin B-6 depletion and increase again during repletion (105, 106, 108, 110). However, the influence of dietary intakes of graded levels of vitamin B-6 on urinary excretion has not been investigated.

The use of urinary excretion of 4-pyridoxic acid measurements in assessing vitamin B-6 status has been criticized because 4-pyridoxic acid can be produced by nonspecific enzymes, and therefore its excretion would not necessarily be related to vitamin B-6 status (32). Recently,

in a short-term metabolic study, 4-pyridoxic acid excretion did not correlate with dietary vitamin B-6 intake, erythrocyte AspAT activity and stimulation by PLP added in vitro, or plasma PLP levels (171).

Traditionally, 4-pyridoxic acid has been measured fluorometrically after extensive sample preparation by ion-exchange chromatography to remove interfering compounds present in the urine (178, 179). Recently, a simple, rapid and sensitive high performance liquid chromatographic method has been developed (180).

Plasma, erythrocyte, and whole blood levels of vitamin B-6 also decrease rapidly during vitamin B-6 depletion and increase after repletion (105, 106, 108), but these measurements have not been successfully used to determine vitamin B-6 status. Vitamin B-6 vitamers traditionally have been measured by microbiological methods using mainly Saccharomyces uvarum (181), although procedures employing Koeckera brevis have been described (182-184). There have been reports that S. uvarum has a greater growth response to pyridoxine than to pyridoxal or pyridoxamine (181, 185-187) and that K. brevis exhibits an even greater disparity (186). For individual vitamin B-6 vitamer measurement, column chromatography is used for separation of the vitamers prior to microbiological determination (185). A recent preliminary report regarding the use of high performance liquid chromatography in measuring vitamin B-6 vitamers in human plasma seems promising (188).

Total vitamin B-6 levels tend to be a poorer indicator of status than plasma PLP levels since individual levels of vitamin B-6 vitamers other than PLP fluctuate considerably (171). Plasma PLP levels are currently considered to be the best measurement of vitamin B-6 status in humans. PLP is the functional form of the vitamin and represents a rapidly mobilizable storage pool of vitamin B-6 as well as the major transport form (163). Animal studies indicate that plasma PLP is derived solely from the liver and that plasma PLP levels correlate well with vitamin B-6 intake and PLP tissue levels, particularly with PLP content of skeletal muscle which is the largest repository of vitamin B-6 in the body (189). In humans plasma PLP remains relatively constant over time (81, 82, 110). When vitamin B-6 dietary intake is altered, plasma PLP levels are changed in accordance with intake and reach new steady-state levels in 3 to 4 weeks (110). The menstrual cycle and chronic use of oral contraceptives does not affect plasma PLP levels in most females (110, 190).

Various enzymatic assays have been used to measure plasma PLP (191-198), but the tyrosine apodecarboxylase procedure is recommended as the method of choice (199). In this procedure the amount of $^{14}\text{CO}_2$ evolved from the PLP-dependent decarboxylation of uniformly labeled ^{14}C -tyrosine in the presence of PLP and tyrosine apodecarboxylase is determined by liquid scintillation spectrometry (191, 192).

Vitamin B-6 Status and Requirement During Pregnancy

Numerous studies have demonstrated that a significant number of pregnant women exhibit a relatively poor vitamin B-6 status as indicated by various biochemical tests. When the vitamin B-6 status of 458 pregnant women was assessed by the in vitro stimulation of erythrocyte AspAT activity by PLP, 40 to 60% were in suboptimal status as compared to a control group of 300 male and female blood donors (114). It was suggested that vitamin B-6 supplementation was required by 50% of the pregnant women studied in order to maintain normal coenzyme saturation of the enzyme. When measuring whole blood PLP levels in 10 pregnant women, Shane and Contractor (113) reported significantly lower levels (5.1 ± 1.3 ng/ml, mean \pm SD) than those of 12 nonpregnant women (9.6 ± 1.7 ng/ml) and 9 oral contraceptive users (7.6 ± 1.1 ng/ml).

Sixty-eight percent of 127 low-income pregnant adolescent and adult women exhibited poor vitamin B-6 status during the first trimester as measured by PLP stimulation of erythrocyte AlaAT activity by in vitro addition of PLP (200). When a subsample was evaluated again at 30 weeks gestation after participation in the Special Supplemental Food Program for Women, Infants and Children (WIC), 63% were in suboptimal vitamin B-6 status. Kaminetzky et al. (201) reported low blood PLP levels in a significant number of 246

pregnant teenagers. All the mothers of low birth-weight infants in that study exhibited low PLP levels.

Contractor and Shane (32) investigated the effect of a 50 mg oral dose of pyridoxine on the blood PLP levels of the mother and fetus as well as nonpregnant women. Initially, the PLP levels of the pregnant women were significantly lower than the nonpregnant controls, indicating a relative vitamin B-6 deficiency. Also, the cord blood PLP levels were significantly higher than the maternal blood levels which suggested active transport of vitamin B-6 across the placenta. Significant differences were also found between the low blood PLP levels of the pregnant women and the higher PLP levels of the nonpregnant women at various times after oral loading with vitamin B-6. These observations could be explained by active transport of the vitamin to the fetus and increased uptake by maternal tissues during pregnancy. These factors are believed to be responsible for the relative vitamin B-6 deficiency observed during pregnancy.

Brin (33) obtained similar results when measuring plasma PLP and erythrocyte AspAT and AlaAT activities in maternal and cord blood. He pointed out that the maternal deficiency could easily result from fetal sequestration of the vitamin since the fetal compartment, which accounts for 10 to 20% of the pregnant woman's physiological mass, can concentrate vitamin B-6 two to threefold. This would be true particularly if the mother has a low dietary vitamin B-6

intake or has marginal vitamin B-6 status at the onset of pregnancy.

The effects of vitamin B-6 supplementation during pregnancy on maternal and fetal plasma PLP levels at term were studied by Cleary et al. (116). Compared with the mean plasma PLP level of 58 nonpregnant women serving as the control group, 4 of 10 pregnant women receiving 10 mg/day of vitamin B-6 and 10 of 13 receiving 2 to 2.5 mg/day had low PLP levels. The cord plasma PLP levels of the pregnant women with adequate plasma PLP levels were compared with the cord levels of mothers who had low plasma PLP levels. The low PLP group had only 53% of the PLP cord concentration of the normal group, indicating that the vitamin B-6 status of the mother significantly affects the plasma PLP levels of the fetus. Hamfelt and Tuvemo (202), measuring plasma PLP levels and in vitro stimulation of erythrocyte AlaAT by PLP, also reported that a minimum of 10 mg/day of vitamin B-6 may be needed to raise maternal and fetal vitamin B-6 levels to normal non-pregnant levels.

By supplementing 26 pregnant women with 2.5, 4 or 10 mg of pyridoxine daily, Lumeng et al. (117) demonstrated that plasma PLP levels in all supplementation groups reached a maximum at 13 to 18 weeks gestation and fell to a minimum at term. All subjects taking 2.5 mg/day of vitamin B-6 and two of the 6 subjects taking 4 mg/day developed low plasma PLP levels during the third trimester, and all had low PLP levels at term. In this study, low plasma PLP levels were

defined as <4.7 ng/ml, the value 2 standard deviations below the mean of a nonpregnant control group. One of the 10 women receiving 10 mg of vitamin B-6 daily had plasma PLP in the deficient range. There was no significant difference in the effect of supplementation between the 2.5 and 4 mg on plasma PLP levels throughout pregnancy. Stimulation of erythrocyte AlaAT and AspAT activity by in vitro addition of PLP was also measured, but these values changed inconsistently with time and did not correlate well with plasma PLP measurements. These results indicate that more than 4 mg of vitamin B-6 daily is required by pregnant women in addition to usual dietary sources to prevent the low levels of plasma PLP indicative of vitamin B-6 deficiency at term.

The relationships between vitamin B-6 intake, vitamin B-6 levels in maternal serum, cord serum and milk was recently investigated in 106 pregnant and lactating women by Roepke and Kirksey (159). The vitamin B-6 intake from unsupplemented diets was less than two-thirds of the RDA during pregnancy. Subjects consuming 2.5 mg/day or less had significantly lower serum vitamin B-6 levels at delivery than those consuming more than 2.5 mg. At delivery the maternal serum vitamin B-6 levels were lower in mothers whose infants had Apgar scores less than seven at one minute after birth than in those whose infants scored seven or better. In assessing the newborn, Apgar scores are determined by numerical values given for heart rate, respiratory effort, muscle tone, reflex irritability, and

color for a maximum score of 10 (203, 204). Maternal serum vitamin B-6 levels at 5 months gestation significantly correlated with vitamin B-6 levels in the cord serum at delivery and in milk 14 days postpartum. Since this stage of gestation precedes the period of rapid growth in the fetal central nervous system, these researchers observed that 5 months gestation is a critical time for assessing maternal vitamin B-6 status.

These data also demonstrate the effect of the vitamin B-6 nutritional status and intake of the mother during pregnancy and lactation on the vitamin B-6 content of the mother's milk. It has been clearly shown that the vitamin B-6 content in human milk responds rapidly to changes in vitamin B-6 intake since there are no apparent regulatory mechanisms to maintain the vitamin B-6 concentration within definite limits (205, 206).

The condition of the infant at birth has also been related to the vitamin B-6 status of the mother during pregnancy by Schuster et al. (200). Infants whose mothers exhibited poor in vitamin B-6 status at around 15 weeks gestation had significantly lower Apgar scores at one minute after birth than those whose mothers exhibited adequate vitamin B-6 status.

Long-term oral contraceptive use has been implicated in compromising the vitamin B-6 status of women during pregnancy and lactation. Roepke and Kirksey (207) reported that vitamin B-6 levels in maternal serum at 5 months

gestation and delivery and in cord serum and milk were lower in the long-term (>30 months) oral contraceptive users than in short-term users or non-users. At 5 months gestation the mean maternal serum level of long-term oral contraceptive users was only 49% of the mean for non-users and 59% of the mean for short-term users. These data suggest that long-term use of oral contraceptives prior to conception may reduce maternal vitamin B-6 reserves.

Serum PLP levels of premature infants have been found to be extremely low (mean = 0.82 ng/ml \pm 0.97 SD) at birth (208). This observation suggests that the most rapid fetal uptake of vitamin B-6 may take place during the last weeks of pregnancy, providing one reason for the dramatic decrease in maternal plasma PLP levels during the last trimester, particularly at delivery. Another factor which may contribute to this phenomenon is the hemodilution which peaks at this stage of pregnancy (209).

Vitamin B-6 nutritional status in pregnancy may also be related to several poorly understood disorders of pregnancy, including morning sickness (nausea and vomiting in early pregnancy), gestational diabetes, and hypertensive disorders of pregnancy such as pre-eclampsia. The majority of pregnant women experience nausea and/or vomiting during the first trimester of pregnancy (210). The interest in a possible relationship between vitamin B-6 and morning sickness dates back 40 years when altered tryptophan metabolism was observed in pregnant women, particularly those with morning

sickness. This led to the inclusion of pyridoxine in antinausea prescription drugs such as Bendectin used to treat the symptoms of morning sickness. Studies dating back 20 to 40 years ago reported conflicting results about the effect of vitamin B-6 therapy for morning sickness (211-215).

In 1974 Reinken and Gant (216) determined the serum PLP levels before and after treatment with 100 mg of pyridoxine daily for 7 days in 24 pregnant women who were experiencing vomiting. Pregnant women who were not experiencing morning sickness had higher levels of serum PLP than the experimental subjects. The serum PLP levels of the experimental subjects in the first trimester were comparable to levels found in healthy pregnant women in the third trimester of pregnancy. After treatment, the serum PLP levels in the subjects increased dramatically. The researchers claim that there was also an alleviation of the clinical symptoms of morning sickness, but no data were presented to substantiate this. In fact, it is hardly surprising that women who experience daily vomiting in contrast to the milder symptoms of morning sickness would be poorly nourished with respect to vitamin B-6 and other nutrients due simply to the significant loss of nutrients through vomiting. Unfortunately, this poorly-designed study has not clarified any possible relationship between vitamin B-6 and morning sickness.

A double-blind study was conducted by Wheatley (217) in 1977 to compare the effects of the antinausea drug Debendox (the British equivalent of Bendectin) with 10 mg of extra pyridoxine with a placebo containing 10 mg of pyridoxine. The Debendox preparation was more effective in decreasing the frequency of nausea and the severity of nausea and retching but was no more effective than pyridoxine in decreasing the severity of vomiting. The significance of these findings is difficult to assess. These data do indicate that Debendox is not effective in treating the most severe symptoms of morning sickness.

Gestational diabetes mellitus is a condition associated with high perinatal mortality rates which becomes manifest during the stress of late gestation. A possible relationship between diabetes and vitamin B-6 was first postulated when a high-tryptophan low-vitamin B-6 diet caused diabetes to develop in rats (218, 219). It was demonstrated that a complex between insulin and the tryptophan metabolite xanthurenic acid was formed which reduced insulin activity. The proposed mechanism is a coupling of the xanthurenic acid at the histadine-zinc group to insulin (220). Xanthurenic acid binds to both albumin and insulin in the serum, and the amount of xanthurenic acid bound to serum protein is greater in normal serum than in diabetic serum (221). The glucose tolerance curves of 13 pregnant women with gestational diabetes in late pregnancy improved after treatment with 100 mg/day of vitamin B-6 for two weeks (222). Insulin levels

remained the same or decreased at all time-points on the two-hour curve, suggesting that the treatment increased the biological activity of the plasma insulin. However, no measurements to determine vitamin B-6 status in these patients were made.

Pre-eclampsia is characterized by hypertension, proteinuria and edema which appear after the 20th week of gestation. It usually occurs in the very young or old primigravidas and can be fatal. Levels of vitamin B-6 vitamers in pre-eclamptic placentae have been reported to be only 40% that of normal placentae (223). Pre-eclamptic newborn had less than 50% the cord blood PLP concentration of normal infants (224). Subjects in a survey of 246 pregnant teenagers who developed pre-eclampsia all exhibited low blood PLP levels (201).

Low fetal plasma PLP levels may result from decreased fetal synthesis of PLP or from decreased placental uptake of maternal PLP. Low activities of two enzymes involved in vitamin B-6 metabolism in pre-eclamptic placentae have been reported. Pyridoxal kinase and pyridoxamine (pyridoxine) phosphate oxidase activities were reduced in pre-eclamptic placentae while phosphatase activity was not (223). The placental uptake of other nutrients which involve active transport such as amino acids has been shown to be decreased in pre-eclampsia (225).

Reports regarding the effect of vitamin B-6 supplementation on the incidence of pre-eclampsia have been

contradictory. Hillman et al. (226) found no significant difference in the incidence of pre-eclampsia when vitamin B-6 alone was given to a low socioeconomic group. However, the incidence of pre-eclampsia was reduced by 50% when vitamin B-6 was added to a general vitamin regime in middle-class subjects (227).

EXPERIMENTAL PROCEDURE

Subjects

The subjects in this study were clients attending Maternal and Infant Care (MIC) clinics located in Alachua, Marion and Putnam counties in north central Florida between June 1981 and April 1983. The MIC clinics are state-funded facilities which provide prenatal and postpartum care for eligible low-income women and their infants. Two hundred and forty women volunteered to participate in the study during their first prenatal clinic visit. The group was 48% black and 52% white. Subsequently, 47 subjects stopped attending the clinic, 3 were not pregnant, 4 miscarried, 4 delivered prematurely, and 65 discontinued taking the vitamin B-6 supplements. Blood samples were obtained from 71 subjects at the 30-week gestation clinic appointment, and 40 maternal and cord blood sample sets were obtained at delivery.

A nonpregnant group of healthy women was used to provide normal nonpregnant values of plasma PLP levels and erythrocyte AspAT activity and stimulation by exogenous PLP. Twenty-seven well-nourished students and staff members at the University of Florida volunteered to provide one 10-ml fasting blood sample. These women, ranging in age from 19 to

34 years, did not take oral contraceptives or vitamin B-6 supplements.

Experimental Design

Subjects were randomly assigned a vitamin B-6 supplement containing 0, 2.6, 5, 7.5, 10, 12.5, 15 or 20 mg of pyridoxine-HCl at their first prenatal clinic visit. Since this was a double-blind study, the supplements were identified by a letter code in order that neither subjects nor researchers knew what level was being administered. The key to the code was not released until the study was finished and all samples were analyzed. The vitamin B-6 supplements were provided by the Hofmann-LaRoche Co. (Nutley, New Jersey). The supplements were in tablet form and contained lactose, microcrystalline cellulose, corn starch, magnesium stearate, and pyridoxine-HCl.

Maternal health and vitamin B-6 status were assessed at three stages of pregnancy: first prenatal clinic visit (<22 weeks gestation), 30 weeks gestation, and at term. Vitamin B-6 status and condition of the infant at birth were also determined. Dietary vitamin B-6 intake of each subject was estimated from a 24-hour dietary recall.

Research Protocol

First Prenatal Clinic Visit

Eligibility of the subjects to participate in the study was determined after completion of their medical history and physical examination. Criteria for eligibility were 1) good health at first visit, 2) less than 22 weeks pregnant, 3) 17 years or older, 4) not taking vitamin B-6 supplements or medications such as Bendectin which contain vitamin B-6, 5) and no long-term history of oral contraceptive use (more than one year within 6 months of current pregnancy).

The purpose and protocol of the study were explained to each eligible client, and each was asked if she wished to volunteer to participate in the study. A study description and instruction form (form 1 in Appendix) was given to each volunteer along with a bottle containing 200 supplements. Each subject was also verbally instructed to take one tablet daily and to bring the bottle with any remaining supplements to the 30-week gestation clinic appointment. Informed consent forms (form 2 in Appendix) were read and signed by the subjects, and a copy was given to each one.

Subjects were interviewed regarding morning sickness (nausea and vomiting) experiences and the use of alcohol, tobacco and other drugs. Other personal and medical data were taken from the subject's medical record, including hematocrit, blood pressure, height, weight, pre-pregnancy weight, age, race, gestational age, and parity. A 10-ml

blood sample was obtained from each subject by the clinic nurse.

Thirty-week Clinic Visit

At 30 weeks gestation, each client attending the prenatal clinic is given a glucose tolerance test to screen for diabetes mellitus. At this appointment a 10-ml blood sample was also obtained from subjects in this study. The subject was interviewed, and a new bottle of vitamin B-6 supplements was provided. Each subject was questioned regarding her degree of compliance with supplementation. In addition, the number of pills remaining in the old bottle of supplements returned by the subject was counted. A 24-hour dietary history was obtained. Medical record data were recorded, including weight, hematocrit, blood pressure, and glucose tolerance.

Delivery

The majority of the subjects delivered at Shands Teaching Hospital on the University of Florida campus, and some of the low-risk clients delivered at Putnam County Hospital or Munroe Regional Medical Center (Marion County) through the midwifery program. A 10-ml blood sample was obtained from each subject during delivery. A cord blood sample was collected after ligation of the cord. Data regarding maternal health status were obtained from the medical record, including hematocrit, blood pressure, and information regarding complications during pregnancy and delivery. Placental weight was also recorded. Information

obtained regarding the condition of the infant at birth included length, weight, Apgar scores at 1 and 5 minutes after birth, and any abnormalities or health problems.

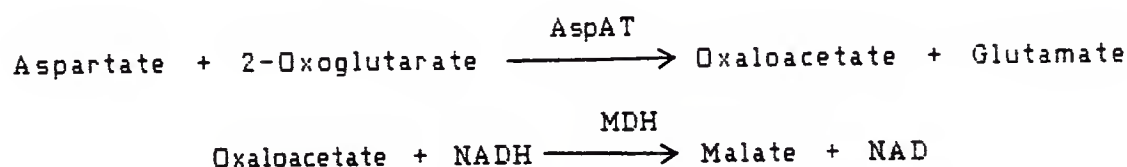
Sample Collection

All blood samples obtained from the subjects were collected by venipuncture in sodium heparinized Vacutainer tubes. Cord blood was also collected in heparinized tubes. The samples obtained at the initial and 30-week visits were immediately centrifuged at approximately 1000 x g for 15 minutes, and the plasma was removed and saved. The erythrocytes were washed in an equal amount of 0.85% saline solution and again centrifuged for 15 minutes. The supernatant was removed, and the erythrocytes transferred to storage vials. Sample handling was carried out in subdued light to minimize destruction of vitamin B-6. All samples were stored on ice in a covered container while transported to the university laboratory and then stored at -30°C until analyzed. Maternal and cord blood samples obtained at delivery were also centrifuged for 15 minutes, and the plasma was transferred to storage vials and immediately frozen.

Biochemical Analyses

Aspartate Aminotransferase Activity

Erythrocyte AspAT activity and in vitro stimulation by exogenous PLP were assayed using a continuous flow procedure developed by Skala et al. (177) with several modifications. This method is based on the following reaction sequence:



where MDH is malic dehydrogenase and NADH and NAD are the reduced and oxidized forms of nicotinamide adenine dinucleotide, respectively.

AspAT activity is proportional to the rate of oxidation of NADH which can be measured by the rate of decrease in absorbance at 340 nm or fluorescence at 470 nm. Lactic dehydrogenase is added to reduce endogenous pyruvate, a source of competing side reactions.

An AutoAnalyzer I (Technicon Instruments, Tarrytown, NY) was used in this study. This system measures the endpoint of the indicator reaction. It was therefore calibrated with a series of control red cell hemolysates, the AspAT activity of which were measured by a kinetic method performed manually which will be described later. These hemolysates served as standards and were included with every 30 samples analyzed by the AutoAnalyzer I system.

The reagents used for the continuous flow method and their concentrations are listed in table 1 and were obtained from Sigma Chemical Co. All reagents except PLP were prepared with the Tris buffer solution and brought to pH 7.8. Triton X-100 was added to the aspartic acid and NADH/MDH solutions to give a 0.1% final concentration. Tris buffer was added to erythrocytes to provide a 1/15 dilution. Erythrocytes were stored at -30°C for 1 - 10 months prior to analysis.

The integration of the reactants into the system is shown in figure 4. The hemolysate samples were incubated with aspartic acid at 30°C. PLP was added at this point to measure in vitro stimulation of AspAT activity by exogenous PLP. Water was substituted for the PLP to measure basal AspAT activity. The reaction was initiated by 2-oxoglutarate, as recommended by the International Federation of Clinical Chemistry (IFCC) (228). The resulting oxaloacetate was dialyzed into the recipient stream. The NADH/MDH solution was introduced and the indicator reaction took place on the recipient side of the dialyzer. After incubation at 30°C for approximately 5 minutes, the NADH concentration was measured fluorometrically (Aminco FluoroMonitor) and recorded using a strip chart recorder. AspAT activity is linearly proportional to the decrease in fluorescence as NADH is oxidized to NAD, which unlike NADH does not fluoresce at 470 nm when excited at 340 nm. A 0

Table 1. Reagent concentrations for continuous flow analysis of AspAT activity.

Reagent	Concentrations	
	Working Reagent	Final Reaction
Tris (hydroxymethyl aminomethane)	104	82 (mM)
L-Aspartate	505	200 (mM)
NADH	0.12	0.12 (mM)
MDH	610	610 (U / l)
2-Oxoglutarate	80	12 (mM)
PLP	0.66	0.10 (mM)
Volume fraction of sample	0.25	(0.025 - 0.04)
pH	7.8	7.8

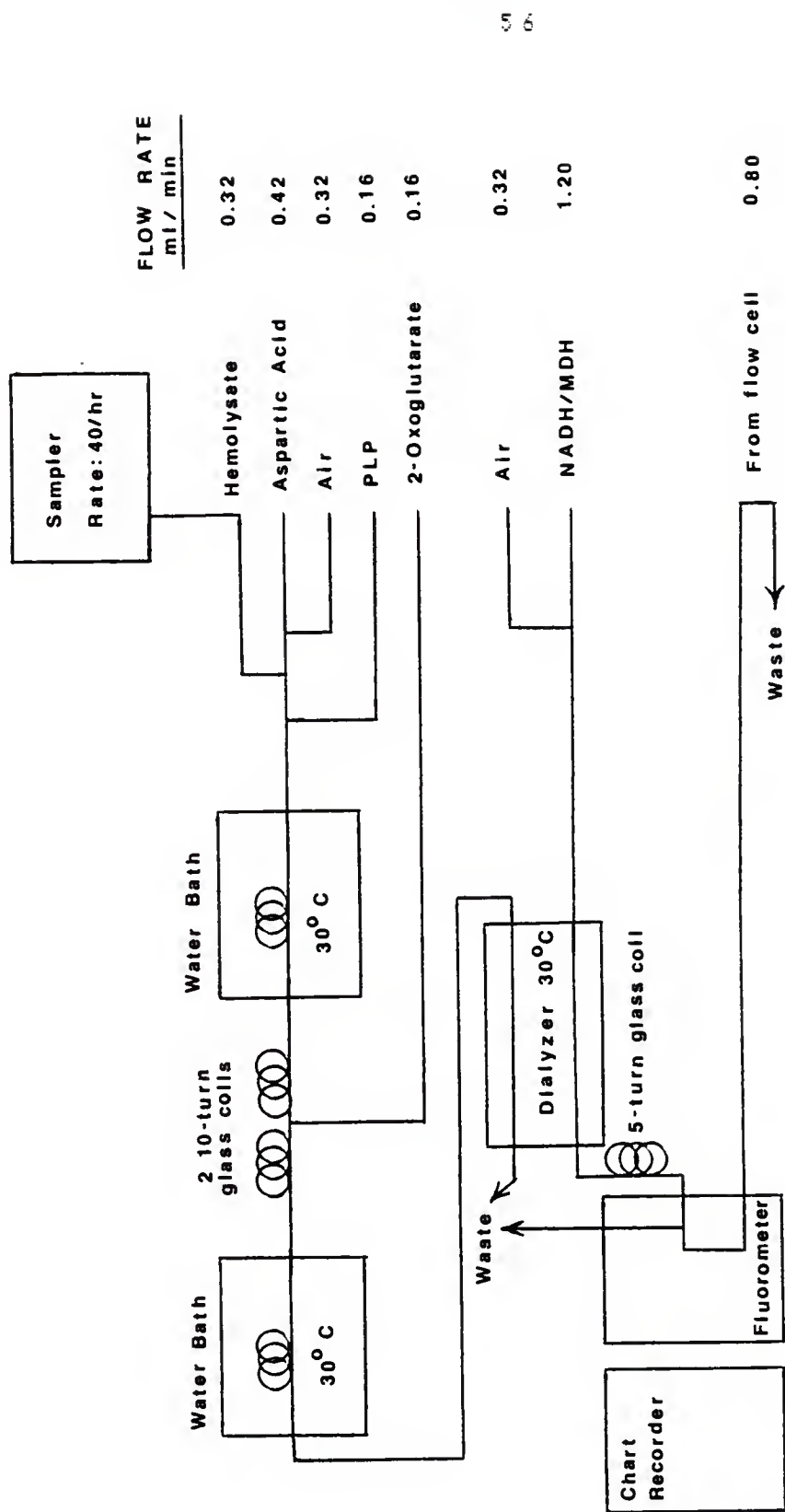


Figure 4. Manifold diagram for continuous flow determination of AspAT activity in hemolysates.

fluorescence baseline was established by substituting water for the NADH entering the system.

The AspAT activities of the hemolysates used as standards to calibrate the continuous flow method were determined manually immediately prior to each series of determinations by the AutoAnalyzer I. Due to insufficient supply of control erythrocytes, different ones were used each time. However, a sufficient amount of one sample allowed for its measurement each time to study the stability of erythrocyte AspAT activity with time under the storage conditions of the study.

The assay conditions used were those optimized and recommended by Bergmeyer et al. (176) for determination of serum AspAT activity. The reagents used and their final concentrations are listed in table 2. The hemolysates were prepared by adding deionized distilled water to thawed erythrocytes to yield a 1/45 final dilution and kept on ice. All samples were analyzed in triplicate. The reagent mixture (2.0 ml) was incubated with the hemolysate (0.2 ml) for 10 minutes at 30°C. Then the reaction was started by adding 2-oxoglutarate (0.2 ml) pre-warmed to 30°C. The reaction was followed spectrophotometrically (Model 250, Gilford Instruments) at 340 nm for 10 minutes. The initial reagent mixture consisted of 1.9 ml Tris buffer, 0.05 ml NADH/MDH solution and 0.05 ml LDH enzyme (from rabbit muscle, Sigma Chemical Co., 340-10) when measuring basal AspAT activity. Stimulation of AspAT activity was accomplished by adding 0.1

Table 2. Assay conditions and final reagent concentrations for spectrophotometric determination of AspAT activity by coupled-enzyme kinetic method.

Temperature	30 °C
pH	7.8
Tris (hydroxymethyl-aminomethane)	80 mM
L-Aspartate	240 mM
2-Oxoglutarate	12 mM
NADH	0.13 mM
MDH	420 U/l
LDH	600 U/l
PLP	0.10 mM
Volume fraction of sample	0.083 (1:12)

ml of PLP solution and decreasing the buffer volume to 1.8 ml.

AspAT activity is expressed in international units per liter of hemolysate and is calculated using the following equation:

$$\text{Activity(U/L)} = \frac{\text{dA/min} \times 2.4 \times 1000 \times \text{TCF}}{6.22 \times 0.20}$$

where dA/min is the change in absorbance per minute, 2.4 is the total reaction volume (ml), TCF is the temperature correction factor which is 1.00 at 30° C, 6.22 is the millimolar absorptivity of NADH at 340 nm, and 0.20 is the sample volume (ml).

The linear range of the assay was determined by analyzing serial dilutions of a control hemolysate. Analysis of another hemolysate stored at -30° C indicated little change (<5%) in AspAT activity over a period of 10 months. AspAT activity was 723.5 U/L with 38% stimulation by added PLP in the first month, 737.9 U/L with 40% stimulation in the fifth month, and 710.2 U/L with 35% stimulation in the tenth month. The mean correlation coefficient (r) of the plots of the standard hemolysates assayed manually versus the continuous flow method was 0.953 as determined by linear regression analysis. This highly significant correlation between the two procedures supports the use of the relatively new continuous flow method for determining AspAT activity in erythrocytes.

Erythrocyte AspAT activity was reported as International Units per gram of hemoglobin (Hb) rather than per liter of red blood cells due to the difficulty in accurately measuring red cell volume. Hemoglobin concentration of the hemolysates was measured manually by the spectrophotometric cyanmethemoglobin method (229). A commercially available kit was used which provided methemoglobin prepared from human hemoglobin as the standard (Sigma Chemical Co. #525-A). Absorbance was determined using a Spectronic 20 spectrophotometer (Bausch and Lomb Co.).

Plasma PLP

Plasma PLP was determined by the method of Lumeng et al. (192) as modified by Sloger and Reynolds (191) which is based on the PLP-dependent decarboxylation of L-tyrosine by tyrosine apodecarboxylase. The apoenzyme was isolated from dried Streptococcus faecalis cells grown in pyridoxine-deficient medium (Sigma Chemical Co. #T4629) and purified to remove contaminating pyridoxal kinase. All steps were carried out at temperatures near 0° C. One gram of cells was suspended in 10 ml cold 0.01 M sodium citrate buffer, homogenized (Con-Torque homogenizer, Eberbach Corp.), and centrifuged at 25,000 x g for 15 minutes at 4° C. The supernatant was saved. The pellet was resuspended in the citrate buffer, and the suspension was sonicated (Sonifier Cell Disruptor, Model W185, Heat Systems Ultrasonic, Inc.) for 7 one-minute periods with one-minute

intervals between periods. The centrifugation and sonification steps were repeated for a total of five times. The pooled supernatant was slowly brought to 60% saturation by adding ammonium sulfate (enzyme grade, Sigma Chemical Co.) with constant stirring. After centrifugation at 25,000 x g at 4° C for 20 minutes, the supernatant slowly was brought to 85% saturation to precipitate the enzyme fraction which was resuspended in 5 ml dialysis buffer. The enzyme suspension was dialyzed (Spectrapor membrane tubing, 12,000-14,000 MW, 25 mm diameter, Fisher Scientific Products) for 12 hours with one change in dialysis buffer after 4 hours. The buffer was a solution of 0.3 M sodium citrate, 24% (v/v) glycerol, and 2mM mercaptoethanol, pH 6.0. The enzyme solution was stored in 1-ml vials at -30°C.

All steps of the plasma PLP assay were performed in subdued light to inhibit photolysis of PLP. The plasma samples were thawed and deproteinized by adding 0.75 ml saline and 0.25 ml trichloroacetic acid to 1 ml of plasma. After mixing with a vortex mixer, the tubes were incubated at 32° C for 15 minutes and centrifuged at 18,000 x g for 15 minutes at 4° C. After transfer of the supernatant to new tubes, centrifugation was repeated. The supernatant was extracted 4 times with water-saturated diethyl ether (purified, Sigma Chemical Co.) using a 4:1 ether to sample volume ratio. Residual ether was evaporated with nitrogen for approximately one hour. The sample extracts were kept on ice until the assay was begun.

After adding 0.1 ml of 0.1 M sodium citrate buffer (pH 6.0), 0.2 ml sample extract, and 0.1 ml apoenzyme (diluted to 0.1 with 0.3 ml sodium citrate buffer) to the reaction tubes (disposable 16x100 mm glass tubes, Fisher Scientific), the reaction mixtures were incubated at room temperature for 30 minutes for holoenzyme formation to occur. All samples were analyzed in triplicate. For the standard curve, 0 - 0.125 ml of the PLP standard solution containing 20 ng/ml were substituted for the sample to provide 0 - 2.5 ng PLP/tube. The amount of buffer was adjusted accordingly. The reaction was begun by adding 1 ml of labelled tyrosine solution to each reaction tube and immediately capping the tubes with rubber stoppers fitted with center wells (Kontes, Inc., #K882320). The wells contained folded chromatography paper (Whatman #1, Fisher Scientific) and 0.030 ml of methylbenzonium hydroxide in methanol (Sigma Chemical Co.) to trap the $^{14}\text{CO}_2$. The labelled tyrosine solution consisted of 50 μCi L- ^{14}C -tyrosine (56 $\mu\text{Ci}/\text{mmol}$, 97.5% pure, Amersham Corp.), 250 ml 0.1 M sodium citrate buffer (pH 6.0), 31 ml 0.15 N HCl, and 0.3763 g L-tyrosine. The reaction continued in a shaking water bath (Thermo-Shake, Forma Scientific Co.) at 32° C and was stopped after exactly 20 minutes by injecting 1 ml of 5 N HCl through the stopper of the tube.

The tubes were kept overnight at room temperature to ensure complete trapping of the $^{14}\text{CO}_2$. The center wells were cut from the stoppers and put into 7-ml polyethylene

scintillation vials. The contents were thoroughly mixed after adding 3 ml of scintillation fluid (Ready-Solv NA, Beckman Instruments). Each sample was counted in a liquid scintillation counter (6892 Series Liquid Scintillation System, Tracor Analytic Inc.) for five minutes. The counting efficiency was 97%. The mean correlation coefficient of all standard curves of the PLP assay was 0.991 as determined by linear regression analysis. The coefficient of variation was 7.2 (n=18) for determination of PLP in plasma using this method. Recovery of PLP added to plasma before deproteinization was 92%.

Analysis of Vitamin B-6 Supplements

The pyridoxine-HCl content of the vitamin B-6 supplements used in this study was determined by the reverse phase high performance liquid chromatographic (HPLC) method developed by Gregory and Kirk (42). The vitamin tablets were manufactured by Hofmann LaRoche Company and consisted of pyridoxine-HCl, lactose, corn starch, magnesium stearate, and microcrystalline cellulose. Three tablets of each concentration were randomly selected from different bottles for analysis. The tablets were dissolved in 100 ml of potassium phosphate buffer, pH 2.2. Each solution was centrifuged at 1000 x g, and the supernatant was filtered through a 0.45 μ m filter before analysis. The HPLC system consisted of an Altex model 312 chromatograph and octadecylsilica column (Partisil 10 ODS-3, Whatman, Inc.).

The column was equilibrated with the mobile phase, 0.033 M potassium phosphate pH 2.2, for 30 minutes before use. Pyridoxine was measured by an Altex ultraviolet analytical detector at 280 nm, and results were recorded on a strip chart recorder. Calibration standards of 25 - 250 µg/ml pyridoxine-HCl (Sigma Chemical Co.) were used. The measured mean pyridoxine-HCl content of the tablets containing 0, 2.6, 5, 7.5, 10, 12.5, 15, and 20 mg was 0, 2.5, 4.9, 7.3, 9.2, 12.1, 14.8, and 20.0 mg.

Dietary Analysis

The dietary information obtained from the 24-hour dietary recalls was analyzed by computer using the Nutrient Dietary Analysis System at Southern Illinois University, Carbondale, Illinois (230). This system was used by the Research Triangle Institute to estimate the dietary intake of pregnant women in a national evaluation of the Special Supplemental Food Program for Women, Infants and Children (WIC).

Statistical Analysis

Vitamin B-6 supplementation levels were plotted against plasma PLP levels and erythrocyte AspAT activity before and after stimulation by exogenous PLP at 30 weeks gestation and at delivery to provide dose response curves. Analysis of variance (ANOVA) procedures were used to examine the effects

of vitamin B-6 supplementation on the various biochemical measurements of vitamin B-6 status of mothers at 30 weeks gestation and at delivery and of the fetus at delivery (231). The effects of such factors as race, parity, tobacco and alcohol use on vitamin B-6 status were also tested by ANOVA. The effect of vitamin B-6 supplementation on infant condition at birth as measured by birth weight, birth length and placental weight were determined by ANOVA procedures. ANOVA was also used to test the effect of vitamin B-6 supplementation level on percent changes in biochemical measurements of maternal vitamin B-6 status between the initial visit and 30 weeks gestation and between the initial appointment and delivery. The Kruskal-Wallis one-way analysis of variance by ranks test was used to examine the effect of vitamin B-6 supplementation on the Apgar scores of infants at 1 and 5 minutes after birth.

Analysis of covariance was used to test the effect of vitamin B-6 supplementation and total vitamin B-6 intake on measurements of vitamin B-6 status at 30 weeks gestation and delivery while accounting for vitamin B-6 status at the first clinic visit (231). Differences in the various indicators of vitamin B-6 status and measurements of pregnancy outcome between subjects who experienced morning sickness and those who did not were tested by the Student's t test (231). This test was also used to compare indicators of maternal vitamin B-6 status measurements of pregnancy outcome between mothers consuming 7.5 mg/day and more

supplemental vitamin B-6 and those taking 5 mg and less. Differences in these parameters between maternal and cord plasma PLP levels above and below the 50th percentile were tested by the Student's t test.

Linear regression methods were used to determine the relationships between plasma PLP levels, erythrocyte AspAT activity and stimulation by exogenous PLP at each sampling time (231). The correlation between maternal and cord plasma PLP levels was also examined by linear regression. Linear regression methods were used to assess the relationship between measurements of vitamin B-6 status and measurements of infant condition at birth. Possible relationships between such factors as age, weight, prepregnancy weight, degree of morning sickness, tobacco and alcohol use and measures of infant condition at birth were also tested by regression analysis. Linear regression methods were used to examine the relationship between the degree of morning sickness experienced by subjects during the first trimester and measurements of vitamin B-6 status, measurements of condition of the infants at birth, parity, age, weight, tobacco and alcohol use. All statistical analyses were performed by computer using the Statistical Analysis System (232).

RESULTS

Nonpregnant Group

The mean values of the biochemical indicators of vitamin B-6 status in twenty-six healthy nonpregnant young women provided "control values" with which to compare the pregnant experimental group of women (table 3). These women did not take vitamin B-6 supplements and had no history of oral contraceptive use. The fasting plasma PLP level was 59.0 ± 19.0 pmol/ml (mean \pm SD), or 14.6 ± 4.7 ng/ml. Plasma PLP values ranged from 28.7 to 117.4 pmol/ml. Mean plasma PLP levels reported in the literature include 37.6 pmol/ml (233), 42.4 pmol/ml (190), and 68.3 pmol/ml (224) for nonpregnant women and 51.8 pmol/ml (233) and 59.8 pmol/ml (234) for men. AspAT activity was 2.08 ± 0.62 U/g hemoglobin and ranged from 1.30 to 3.79 U/g. The mean percent stimulation of AspAT activity by exogenous PLP was $21.6 \pm 15.7\%$ with a 0 - 47.8% range.

Linear regression analysis revealed no correlation between plasma PLP values and erythrocyte AspAT activity or stimulation by added PLP. A negative correlation ($p < .0005$) was found between AspAT activity and percent stimulation by

Table 3. Biochemical measurements of vitamin B-6 status of nonpregnant comparison group and pregnant subjects (mean \pm SD).

	Nonpregnant Women (n=26)	Pregnant Subjects (n=196)
Plasma PLP (pmol/ml)	59.0 \pm 19.0 *	37.1 \pm 25.3 *
Erythrocyte AspAT activity (U/g Hb)	2.08 \pm 0.62	2.63 \pm 1.57
Stimulation of AspAT by PLP (%)	22 \pm 16 **	86 \pm 140 **

Means with asterisks are significantly different ($p < 0.0001$).

exogenous PLP which indicates that lower AspAT activity was associated with insufficient coenzyme.

Since plasma PLP is considered to be the most sensitive indicator of vitamin B-6 status, this measurement was used to compare the pregnant women with the nonpregnant group. The distribution curve of these plasma PLP values was highly skewed to the right (skewness = 1.15); therefore, plasma PLP values were converted to their base 10 logarithm to normalize the distribution curve (235). The mean and standard deviation of the log values were determined. The lowest normal value for the nonpregnant group was defined as the mean minus 2 standard deviations which was 31.5 pmol/ml (7.8 ng/ml). Lumeng et al. (190) used the same procedure to normalize data by log conversion of plasma PLP values prior to calculating the mean minus 2 standard deviations. The value reported by Lumeng et al. (190) using this technique with a group of nonpregnant women was 19.0 pmol/ml (4.7 ng/ml) compared with 31.5 pmol/ml (7.8 ng/ml) in the present study.

Pregnant Group

Description of Subjects

The 196 pregnant women who served as subjects in this study ranged in age from 17 to 38 years. Fifty-two percent of the group was white and 48% black. The mean gestational age at the initial prenatal clinic visit was 15 ± 4 weeks

and ranged from 6 to 21 weeks. Twenty-two percent of the women had no previous pregnancies, 27% had 1, 26% had 2, and 25% had 3 to 7 previous pregnancies. Fifty-three percent reported no tobacco use at the time of the initial appointment, and 85% claimed no consumption of alcoholic beverages. The mean hematocrit for the group was $37 \pm 3\%$, and values ranged from 30 to 49%.

Nutrient Intake

Nutrient intakes were computed for 65 subjects from 24-hour dietary recalls at or near 30 weeks gestation (table 4). The mean dietary vitamin B-6 intake was 1.43 ± 1.28 mg/day (mean \pm SD) which is 55% of the 1980 RDA for vitamin B-6. Eighty-three percent of the subjects consumed less than the RDA.

The mean daily energy intake was 2152 ± 843 kcal which represents 94% of the RDA for women over 23 years old. Values ranged from 885 to 4764 kcal. The mean protein intake was 81.7 ± 38.6 g (122% of the RDA for women over 19 years) which ranged from 21 to 197 g. When expressed as ratios of the vitamin to protein and energy intake, the mean vitamin B-6 intake was 18.9 ± 19.3 μ g/g protein and 0.67 ± 0.63 mg/1000 kcal.

Vitamin B-6 Status at Initial Clinic Visit

The means of the biochemical measurements of vitamin B-6 status for the 196 pregnant subjects determined at the initial clinic visit are compared with the mean values of the nonpregnant group in table 3. The mean plasma PLP level

Table 4. Nutrient intakes of pregnant subjects computed from 24-hour dietary recalls.

Nutrient	Intake (mean \pm SD)	Mean intake as % RDA
Vitamin B-6	1.43 \pm 1.28 mg	55%
Protein	81.7 \pm 38.6 g	122%
Energy	2152 \pm 843 kcal	94%
Vitamin B-6/protein	18.9 \pm 19.3 μ g/g	54%
Vitamin B-6/energy	0.67 \pm 0.63 g/kcal	59%
Vitamin A	1333.9 \pm 1545.2 μ g	133%
Vitamin D	5.79 \pm 5.63 μ g	58%
Vitamin E	13.6 \pm 12.5 mg	136%
Vitamin C	162.0 \pm 142.9 mg	203%
Folacin	329.0 \pm 267.0 μ g	41%
Niacin	22.8 \pm 16.3 mg	152%
Riboflavin	2.53 \pm 1.03 mg	169%
Thiamin	1.61 \pm 1.07 mg	115%
Vitamin B-12	3.9 \pm 3.6 μ g	98%
Calcium	963.5 \pm 683.2 mg	80%
Phosphorus	1181.0 \pm 653.3 mg	98%
Iron	17.9 \pm 13.4 mg	-
Magnesium	224.0 \pm 149.4 mg	50%
Zinc	9.2 \pm 5.4 mg	37%

of the pregnant subjects at the initial prenatal appointment was 37.1 ± 25.3 pmol/ml (9.2 ± 6.3 ng/ml, $n=196$) which, although significantly lower ($p<0.0001$) than the mean of the nonpregnant comparison group, is not considered abnormally low as defined previously (<31.5 pmol/ml). However, 44% of the group did have plasma PLP levels below 31.5 pmol/ml. The mean erythrocyte AspAT activity for the group was 2.63 ± 1.57 U/g hemoglobin ($n=178$), and mean percent stimulation with exogenous PLP was $86\% \pm 140\%$.

Parity, race, tobacco smoking, and alcohol use had no effect ($p>0.05$) on plasma PLP levels, erythrocyte AspAT activity or stimulation by added PLP measured at the initial prenatal visit. When subjects were categorized as "low" or "adequate" on the basis of plasma PLP levels greater or less than 31.5 pmol/ml at the initial clinic visit, there were no differences between groups in age, weight, prepregnancy weight, parity, blood pressure, degree of morning sickness, and hematocrits determined at the initial visit. No significant differences between these groups were found in maternal plasma PLP levels at 30 weeks gestation or delivery, cord plasma PLP levels, birth weight, birth length, placental weight, or Apgar scores at 1 or 5 minutes after birth. The mean gestational age (stage of pregnancy) of subjects in the low plasma PLP group was significantly higher (16 ± 4 weeks) than those with adequate plasma PLP levels at the initial appointment (14 ± 4 weeks, $p<0.02$).

Morning sickness occurs in the majority of pregnant women (210), and the relative vitamin B-6 deficiency observed during pregnancy has led to an interest in the relationship between vitamin B-6 status and morning sickness. Therefore, efforts were made to detect any relationship between vitamin B-6 status and the degree of morning sickness experienced in early pregnancy by the subjects in this study. At the initial prenatal visit subjects were questioned regarding their morning sickness experiences, and the following categories were used to rank the degree of morning sickness: 1) none, 2) mild nausea, 3) occasional nausea and vomiting, and 4) daily nausea and vomiting. These findings are summarized in table 5. Thirty-three percent of the subjects were in category 1, 30% in 2, 22% in 3, and 15% in 4. There was no correlation between any measurements of vitamin B-6 status at the initial clinic visit and the degree of morning sickness experienced by this group in early pregnancy. Women who experienced morning sickness had a greater number of previous pregnancies (2.0 ± 1.6) than those who had no morning sickness symptoms (1.4 ± 1.4 , $p < 0.05$). The placental weights of mothers who had morning sickness were higher (638 ± 123 g) than those who did not (517 ± 94 g, $p < 0.002$). There were no differences in the following parameters between women who did and did not experience morning sickness: plasma PLP levels, erythrocyte AspAT activity or stimulation by PLP, age, weight, prepregnancy weight, blood pressure,

Table 5. Comparison of various measurements of vitamin B-6 status and infant condition at birth and parity between pregnant women who did (Group 1) or did not (Group 2) experience morning sickness in early pregnancy.

	Group 1 (mean±SD)	Group 2 (mean±SD)	
Plasma PLP (pmol/ml)	39.3±28.0 (n=119)	32.6±19.8 (n=55)	
AspAT activity (U/g Hb)	2.73±1.72 (n=109)	2.39±1.14 (n=48)	
Stimulation of AspAT activity (%)	89±163 (n=108)	81±65 (n=47)	
Parity (No.)	2.0±1.7 * (n=84)	1.4±1.4 * (n=42)	p < 0.05
Birth weight (g)	3289±553 (n=84)	3179±466 (n=43)	
Birth length (cm)	50.8±2.8 (n=37)	50.6±2.2 (n=29)	
Apgar score, 1 min	7.7±2.2 (n=75)	8.3±1.0 (n=38)	
Apgar score, 5 min	8.7±1.4 (n=75)	9.0±0.6 (n=38)	
Placenta weight (g)	638±123 ** (n=21)	517±94 ** (n=18)	p < 0.002

Means with asterisks are significantly different.

hematocrit, tobacco or alcohol use, birth weight, birth length, or Apgar scores at 1 and 5 minutes after birth.

Effect of Vitamin B-6 Supplementation on Vitamin B-6 Status

As described in the Experimental Methods section, blood samples were obtained from 71 subjects participating in the study at 30 weeks gestation, and maternal and cord blood samples were obtained from 40 subjects at delivery. Only those subjects who complied with the supplementation protocol as determined by questioning the subjects, counting remaining vitamin B-6 tablets at the 30 week appointment, and obtaining information from the attending nurses and doctors were included in final analysis of the data. This resulted in a final total of 46 subjects at 30 weeks gestation and 22 at delivery. Sixteen of these subjects provided both 30-week and delivery samples. The final number of subjects included within each supplementation group varied according to the parameter measured and is indicated in tables by the designation "n". The 12.5 mg vitamin B-6 supplementation group was excluded from final data analysis because an insufficient number of subjects complied with supplementation protocol (n=3 at 30 week appointment and n=1 at delivery). The total daily vitamin B-6 intake for the subjects was determined by adding the total dietary vitamin B-6 intake of the entire experimental group (1.43 mg/day) to each vitamin B-6 supplement level. The limitations of the 24-hour dietary recall method do not permit using the

estimate of the dietary intake of each supplementation group since the sample numbers are too small (156, 157).

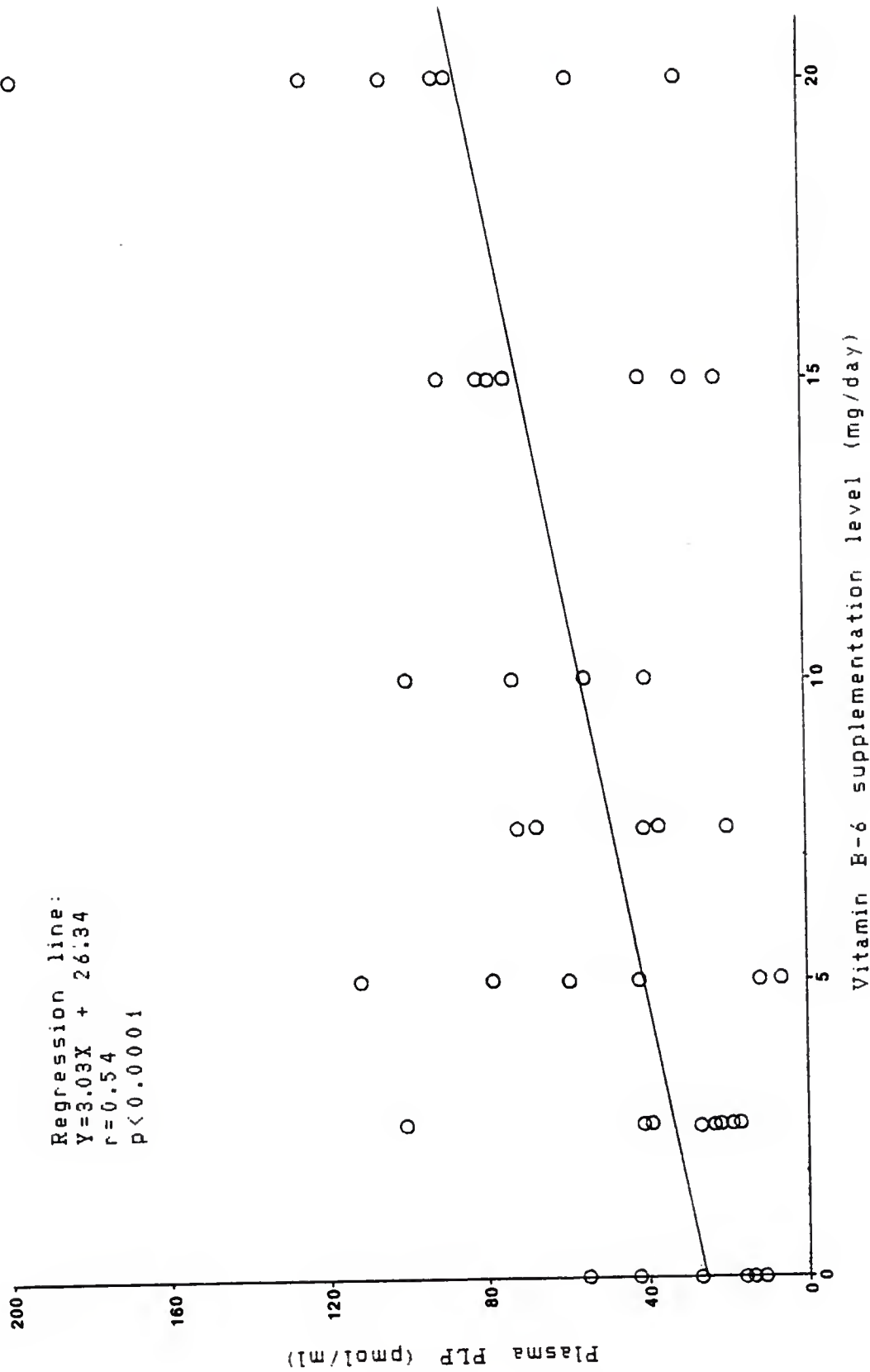
The mean values of the biochemical indicators of vitamin B-6 status for each supplementation group are shown in table 6. The biochemical measurements at the initial prenatal clinic visit were not significantly different ($p>0.05$) among supplementation groups. In this study, erythrocyte AspAT activity and stimulation by exogenous PLP did not correlate with vitamin B-6 supplementation level and were not linearly responsive to graded levels of vitamin B-6 intake. Analysis of covariance with the initial visit AspAT activity and stimulation by PLP as covariables indicated that there was no significant difference in these two measurements among supplementation groups at 30 weeks gestation.

Maternal plasma PLP levels, however, were positively correlated with vitamin B-6 supplementation at 30 weeks gestation ($r=0.55$, $p<0.0005$) and at delivery ($r=0.54$, $p<0.01$). Analysis of covariance with initial visit plasma PLP levels as the covariable indicate that vitamin B-6 supplementation significantly affected plasma PLP levels. There were no significant differences in maternal plasma PLP levels at delivery or in cord plasma PLP levels among supplementation groups. The linear responses of maternal plasma PLP levels at 30 weeks gestation ($r=0.54$, $p<0.0001$) and delivery ($r=0.55$, $p<0.007$) to vitamin B-6 supplementation levels are shown in figures 5 and 6,

Table 6. Biochemical indices of vitamin B-6 status for each supplementation group (mean \pm SD).

	Supplementation Level (Pyridoxine-HCl, mg/day)						
	0 (1.4)*	2.6 (3.5)	5 (5.5)	7.5 (7.6)	10 (9.6)	15 (13.7)	20 (17.8)
Plasma PLP (pmol/ml) (initial visit)	38.8 \pm 17.3 (n=7)	50.0 \pm 32.5 (n=10)	31.4 \pm 10.0 (n=6)	37.3 \pm 6.2 (n=6)	25.8 \pm 13.5 (n=5)	32.5 \pm 31.3 (n=5)	28.2 \pm 12.8 (n=7)
AspAT activity (U/g Hb) (initial visit)	2.86 \pm 1.27 (n=5)	2.61 \pm 1.01 (n=7)	3.09 \pm 0.88 (n=4)	2.59 \pm 0.57 (n=6)	2.07 \pm 0.67 (n=3)	2.01 \pm 0.72 (n=5)	2.47 \pm 1.21 (n=4)
% Stimulation of AspAT (initial visit)	69 \pm 87 (n=5)	60 \pm 39 (n=7)	53 \pm 63 (n=4)	51 \pm 45 (n=6)	124 \pm 76 (n=3)	124 \pm 86 (n=5)	35 \pm 8 (n=4)
Plasma PLP (pmol/ml)	27.5 \pm 17.4	34.8 \pm 26.3	46.9 \pm 39.7	47.7 \pm 22.0	66.7 \pm 25.5	59.9 \pm 28.0	99.6 \pm 52.9
AspAT activity (U/g Hb) (30 wk visit)	3.35 \pm 2.23 (n=4)	3.42 \pm 1.00 (n=6)	---	4.35 \pm 0.92 (n=3)	---	4.50 \pm 1.29 (n=5)	4.25 \pm 0.51 (n=3)
% Stimulation of AspAT (30 wk visit)	31 \pm 10 (n=4)	27 \pm 18 (n=6)	---	11 \pm 11 (n=3)	---	32 \pm 24 (n=5)	0 \pm 0 (n=3)
Plasma PLP (pmol/ml) (maternal at delivery)	8.8 \pm 4.4 (n=4)	18.2 \pm 12.4 (n=4)	12.3 \pm 4.9 (n=3)	46.2 \pm 13.1 (n=3)	24.4 \pm 4.2 (n=2)	31.3 \pm 23.7 (n=2)	48.9 \pm 37.4 (n=4)
Cord Plasma PLP (pmol/ml)	73.6 \pm 49.8 (n=4)	87.1 \pm 32.2 (n=4)	97.9 \pm 55.0 (n=3)	148.1 \pm 8.3 (n=3)	186.5 \pm 67.1 (n=2)	164.2 \pm 67.1 (n=2)	134.6 \pm 73.9 (n=4)

* Total vitamin B-6 intake (diet plus supplement as pyridoxine equivalents).



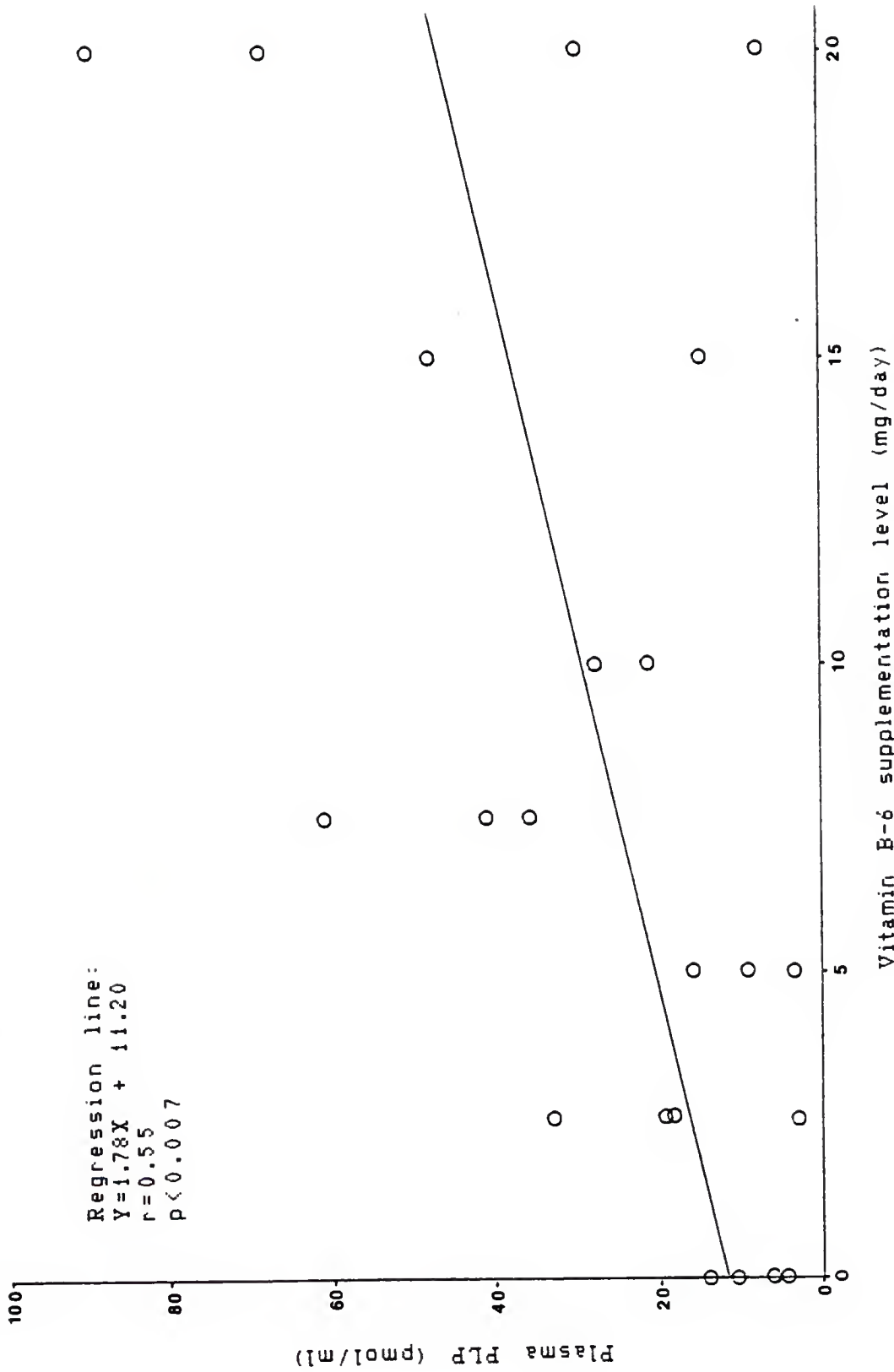
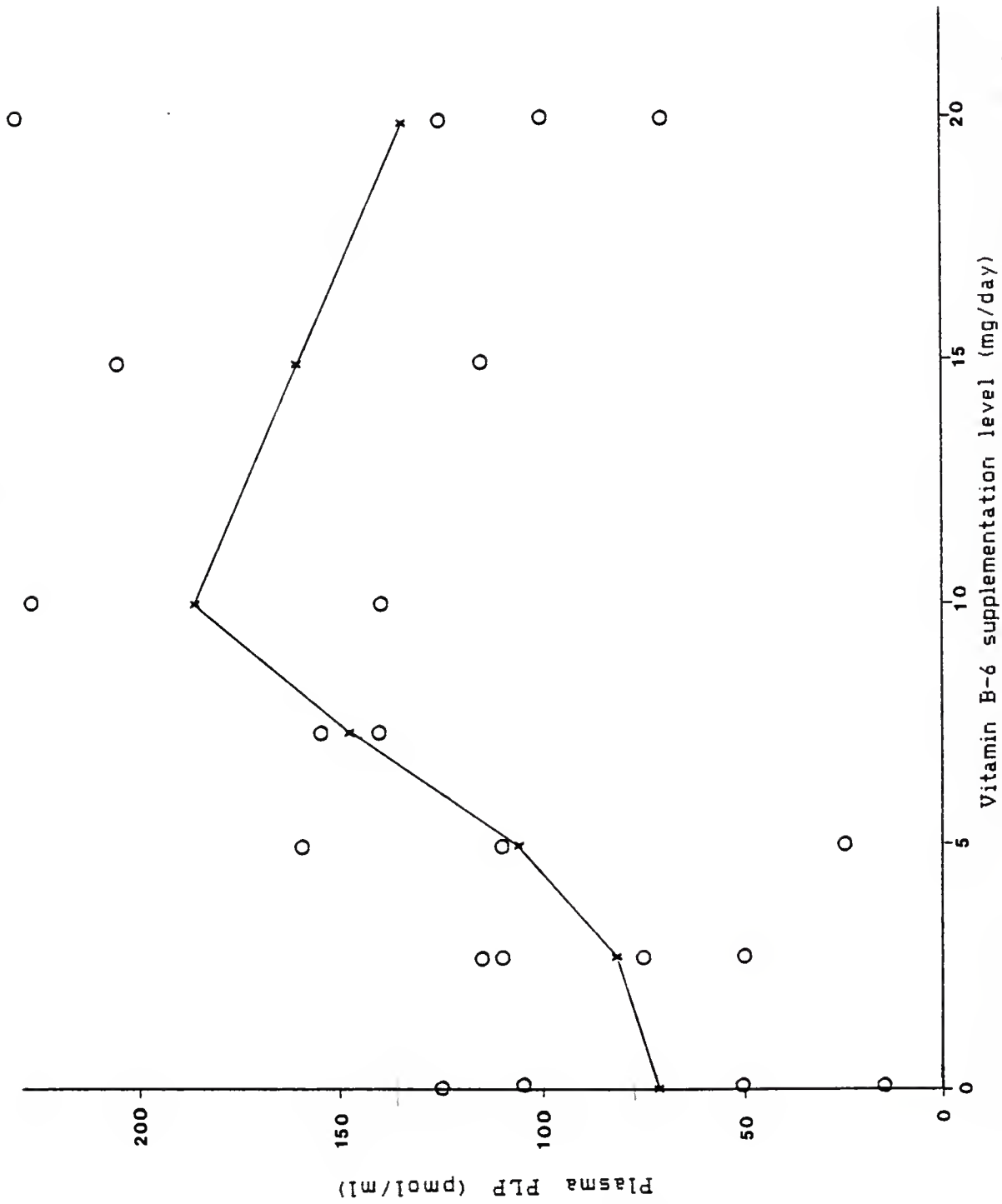


Figure 6. Plot of maternal plasma PLP levels at delivery versus vitamin B-6 supplementation level.

respectively. The response of cord plasma PLP levels to vitamin B-6 supplementation, however, was not linear, as can be seen in figure 7. The cord plasma PLP level increases rapidly until about the 7.5 mg supplementation level when the response leveled off.

The effect vitamin B-6 supplementation on the maternal plasma PLP levels over time and on cord plasma PLP is shown in figure 8. In order to compare the effects of the various levels of supplementation more easily, figure 9 represents the same bar graph after all PLP levels have been adjusted in order that all initial maternal plasma PLP levels are equal. It is apparent that response of maternal plasma PLP at 30 weeks gestation and delivery and cord plasma PLP is essentially the same whether the mother is taking a placebo (0 mg) or 2.6 mg of supplemental pyridoxine-HCl. Neither of these levels is able to prevent a decrease of approximately 30% in maternal plasma PLP levels at 30 weeks gestation, and mean maternal plasma PLP levels at delivery are less than half the levels present at the initial clinic visit in early pregnancy in both supplementation groups. In contrast, all other supplementation levels (5 mg and above) were able to increase the 30 week plasma PLP levels above the initial values. Supplementation with 5 and 7.5 mg of pyridoxine-HCl resulted in an increase of approximately 25% while 10 and 15 mg resulted in an increase of approximately 100%. The most dramatic effect was by the 20 mg supplement which resulted



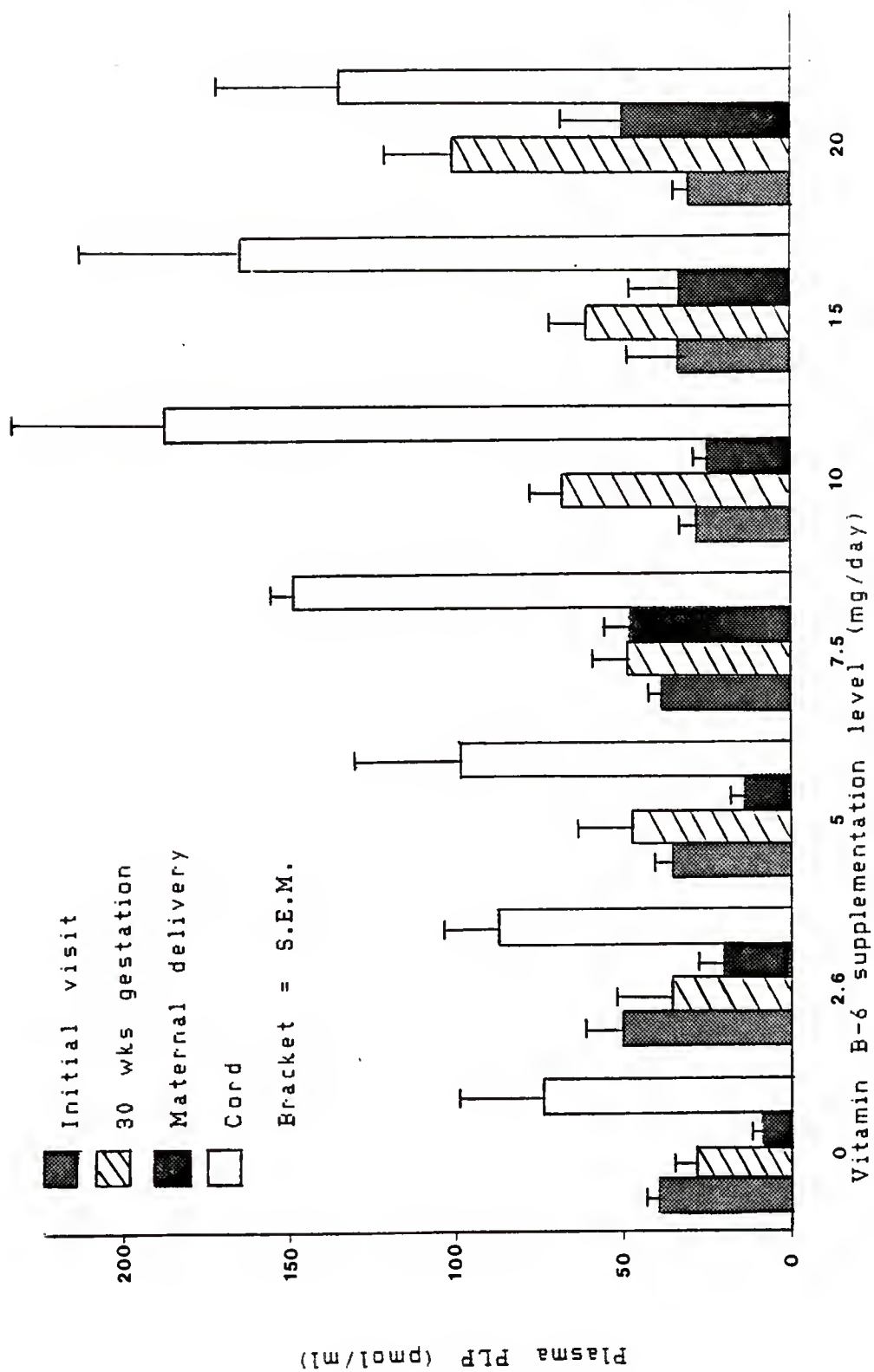


Figure 8. Mean plasma PLP levels of each vitamin B-6 supplementation group.

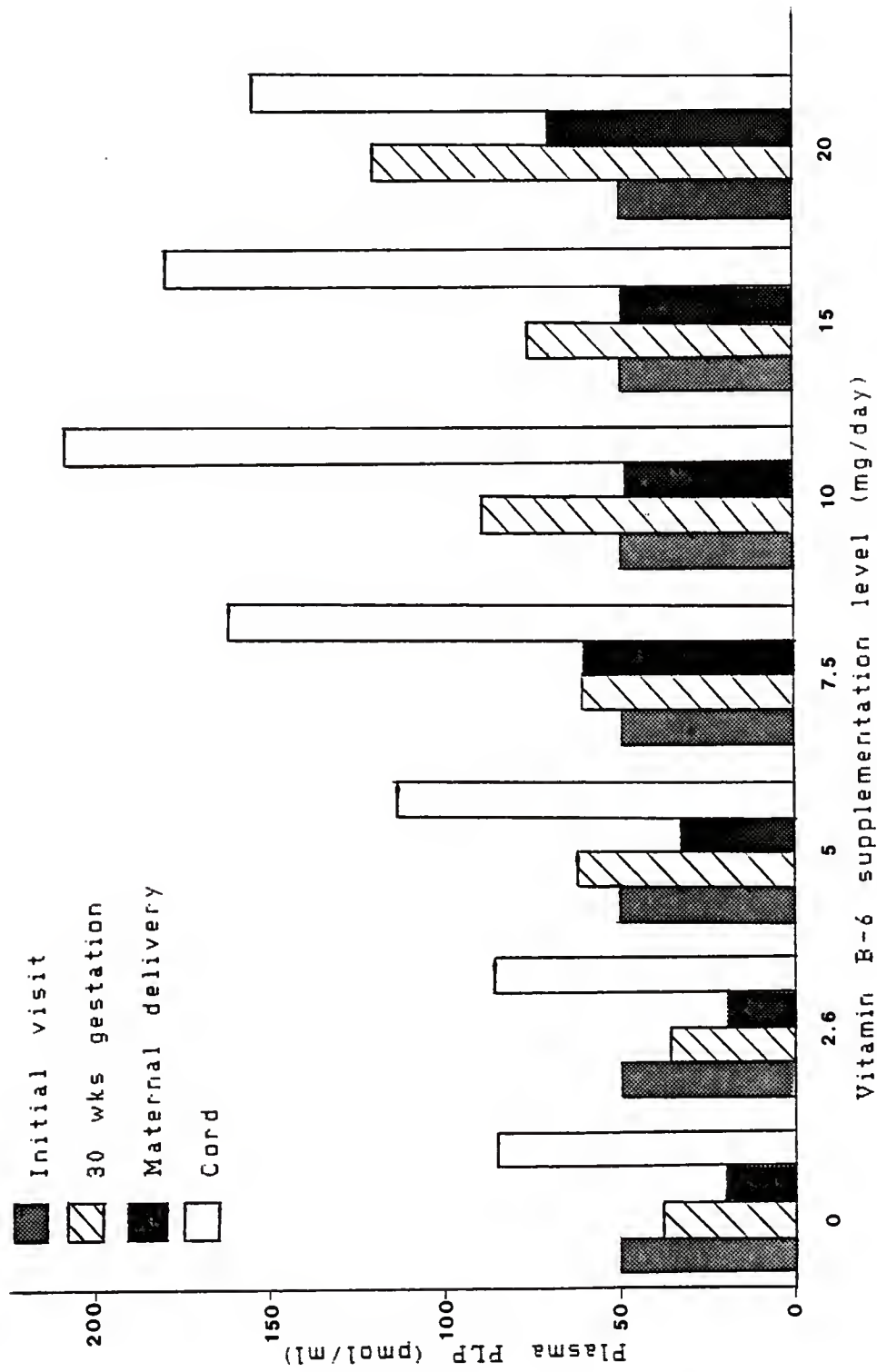


Figure 9. Same as figure 8 when plasma PLP means of supplementation groups are adjusted in order that all initial plasma PLP levels are equal.

in a four-fold increase of 30 week plasma PLP levels above initial values.

Five milligrams of supplemental pyridoxine-HCl or 5.5 mg total maternal intake of vitamin B-6 (diet plus supplement as pyridoxine equivalents) was able to maintain the 30-week plasma PLP at a level comparable to initial values. However, 5 mg of supplemental pyridoxine-HCl did not prevent a decrease of more than 60% in maternal plasma PLP level at delivery from the initial values. The 7.5 mg and higher supplemental levels were able to prevent this decrease. This finding corresponds with the observation that fetal plasma PLP levels appeared to saturate at 7.5 mg supplemental pyridoxine-HCl (figure 7).

Changes in the biochemical measurements of vitamin B-6 status within each subject between the initial visit and 30 weeks gestation and/or delivery were calculated as percentages, and the means within each supplementation group are shown in table 7. Analysis of variance procedures indicated that vitamin B-6 supplementation had a significant effect ($p < 0.01$) on the change in percent stimulation of erythrocyte activity by exogenous PLP between the first visit and 30 weeks gestation (figure 10). However, no statistically significant effect was found in changes in erythrocyte AspAT activity between the first visit and 30 weeks gestation. Vitamin B-6 supplementation significantly affected the percent changes in maternal plasma PLP levels

Table 7. Percent change in biochemical measurements of maternal vitamin B-6 status between initial prenatal appointment and 30 weeks gestation and between initial visit and delivery (mean±SD).

Vitamin B-6 Supplementation level (mg/day)	AspAT 1st visit vs 30 wk (%)	% Stimulation 1st visit vs 30 wk (%)	Plasma PLP 1st visit vs 30 wk (%)	Plasma PLP 1st visit vs delivery (%)
0	17.6±73.7 (n=3)	94.7±51.3 (n=3)	-34.7±24.9 (n=6)	-72.0±11.5 (n=4)
2.6	34.5±85.2 (n=4)	35.1±55.0 (n=4)	-12.3±49.0 (n=9)	-22.5±63.7 (n=4)
5	17.8±21.9 (n=2)	-76.0±5.0 (n=2)	66.0±111.2 (n=5)	-22.5±63.7 (n=3)
7.5	80.3±12.2 (n=3)	-28.9±9.3 (n=3)	40.3±62.1 (n=5)	14.3±36.0 (n=3)
10	---	---	191.3±99.3 (n=4)	-5.8±10.4 (n=2)
15	149.9±78.1 (n=4)	-98.8±1.7 (n=4)	92.0±64.7 (n=7)	43.7±121.6 (n=4)
20	141.0±108.1 (n=3)	-100.0±0 (n=3)	358.1±307.0 (n=7)	144.8±155.2 (n=4)

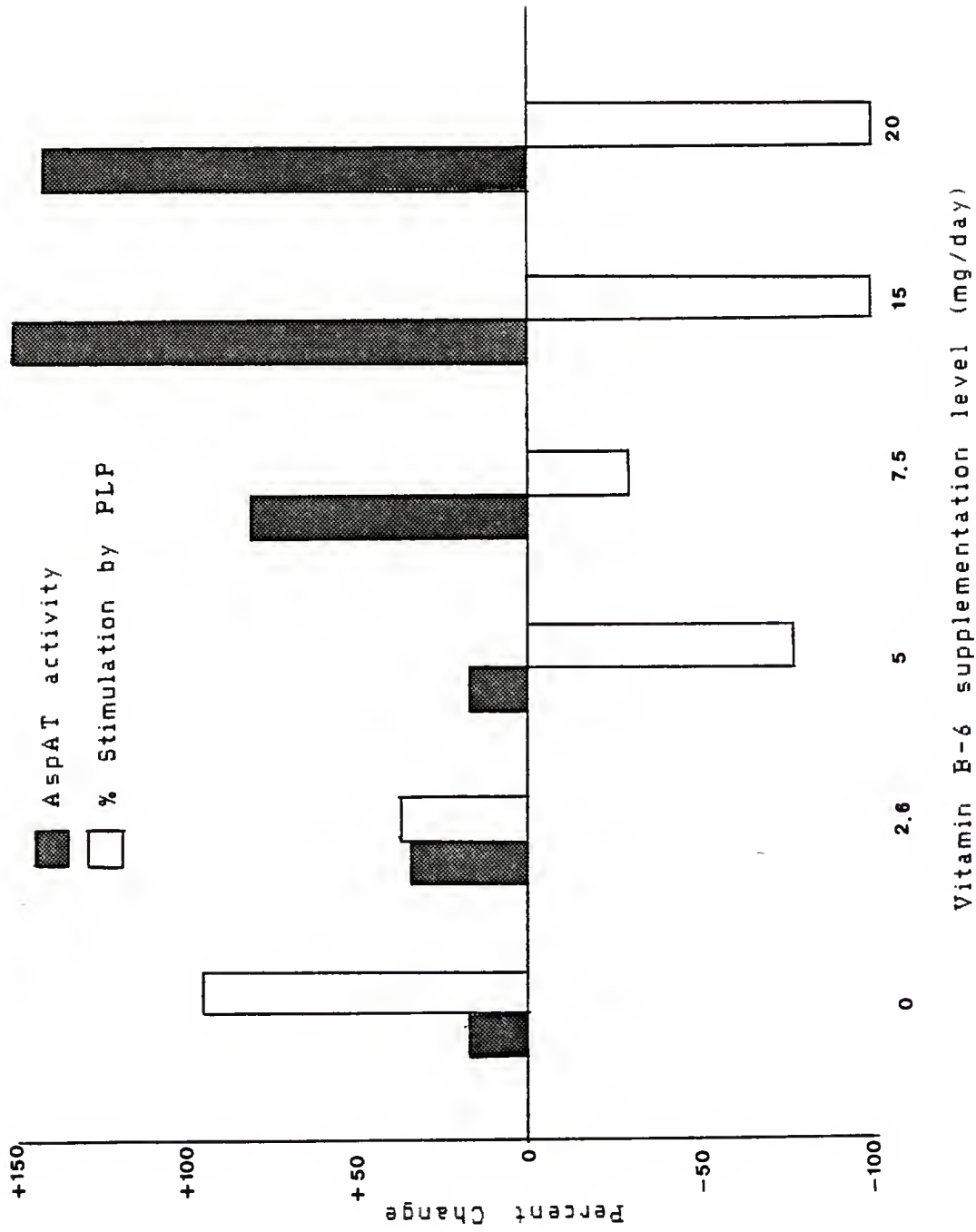


Figure 10. Percent change in erythrocyte AspAT activity and in % stimulation by PLP between the first prenatal appointment and 30 weeks gestation.

between the first and 30 weeks gestation ($p < 0.01$) but not between the first visit and delivery (figure 11).

When plasma PLP levels measured at 30 weeks gestation were classified as "low" (< 31.5 pmol/ml) or "adequate" (≥ 31.5 pmol/ml), there were no significant differences between these groups in hematocrit, maternal or cord plasma PLP levels at delivery, or measures of pregnancy outcome. At delivery, fetal plasma PLP levels were significantly lower from mothers who had low levels of plasma PLP than fetal levels from mothers with adequate PLP levels (83.5 versus 154.4 pmol/ml, respectively; $p < 0.01$). Maternal and fetal plasma PLP levels at delivery were positively correlated ($r = 0.52$, $p < 0.002$). This relationship has been reported previously and confirms that fetal dependence upon the mother for vitamin B-6. Analysis of covariance with initial maternal plasma PLP levels as the covariable showed no differences in ratios of maternal and cord plasma levels among the supplementation groups.

Analysis of variance procedures revealed no differences in measurements of infant condition at birth which included birth weight and length, placental weight, and Apgar scores at 1 and 5 minutes after birth among the supplementation groups. Since 7.5 mg of maternal pyridoxine-HCl supplementation during pregnancy appeared to saturate the plasma PLP of the fetal-placental unit, the condition of infants at birth whose mothers took 7.5 mg or more was compared with that of infants whose mothers took 5 mg or

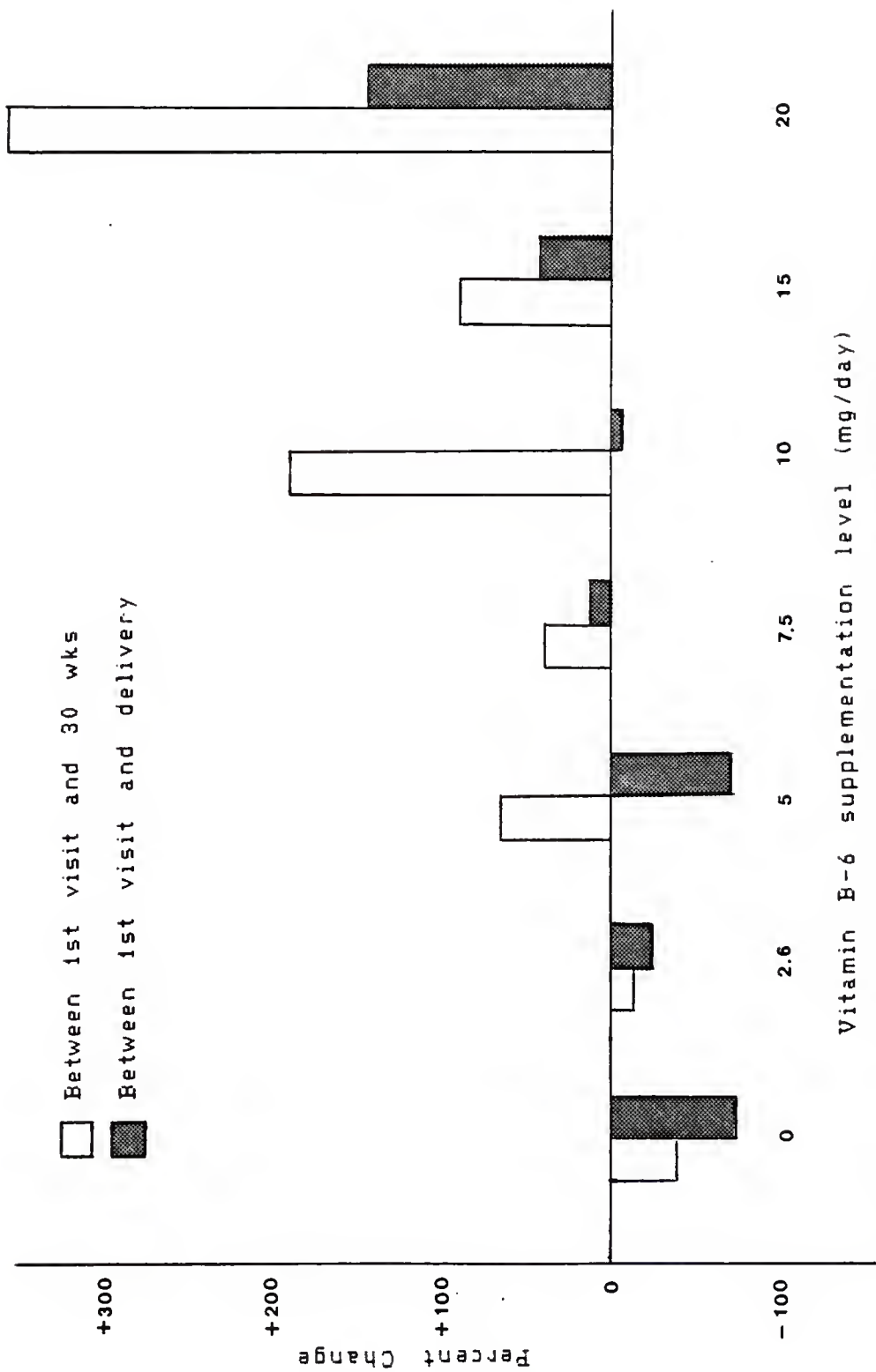


Figure 11. Percent change in plasma PLP levels between the initial prenatal visit and 30 weeks gestation and between the first visit and delivery.

less supplemental pyridoxine-HCl. The means of the indicators of birth outcome and vitamin B-6 status at delivery are shown in table 8. Both maternal and cord plasma PLP levels were significantly higher ($p < 0.005$) when supplementation was 7.5 mg. Apgar scores at 1 minute after birth were significantly higher ($p < 0.05$) for infants whose mothers took 7.5 mg or more supplemental pyridoxine-HCl than for infants of mothers who took 5 mg or less. No effect of maternal vitamin B-6 status at 30 weeks gestation or delivery on infant condition at birth was demonstrated when subjects were categorized as having "low" or "adequate" plasma PLP levels. The means of the measurements of the infant condition at birth for maternal plasma PLP levels at delivery and cord plasma PLP levels grouped above or below the 50th percentile are presented in table 9. The infant Apgar scores at 1 minute after birth were significantly higher ($p < 0.01$) when maternal plasma PLP levels at delivery and cord plasma PLP levels were in the upper 50th percentile. There were no significant differences between the two groups in birth weight, birth length, and placental weights. Other factors such as race, parity, alcohol or tobacco use did not affect the condition of the infant at birth. The frequency of specific Apgar scores in each supplementation group is shown in table 10.

Incidence of Gestational Diabetes and Pre-eclampsia

Only three of the original enrollees in the study developed gestational diabetes during the course of

Table 8. Measurements of vitamin B-6 status at delivery and infant condition at birth when daily vitamin B-6 supplementation was ≤ 5 mg or ≥ 7.5 mg.

	Vitamin B-6 Supplementation Level	
	0-5 mg/day	7.5-20 mg/day
Maternal plasma PLP at delivery (pmol/ml)	12.3 \pm 9.0* (n=11)	38.3 \pm 25.0* (n=12) p < 0.005
Cord plasma PLP (pmol/ml)	85.1 \pm 45.5** (n=11)	152.7 \pm 54.3** (n=11) p < 0.005
Birth weight (g)	3240 \pm 505 (n=24)	3287 \pm 429 (n=26)
Birth length (cm)	50.9 \pm 2.7 (n=16)	50.7 \pm 2.1 (n=17)
Placenta weight (g)	627 \pm 140 (n=11)	548 \pm 114 (n=8)
Apgar score, 1 min	7.6 \pm 2.3*** (n=23)	8.6 \pm 0.6*** (n=24) p < 0.05
Apgar score, 5 min	8.8 \pm 0.8 (n=23)	9.1 \pm 0.6 (n=24)

Means with asterisks are significantly different.

Table 9. Measurements of infant condition at birth when maternal plasma PLP levels at delivery and cord plasma PLP levels were grouped above or below the 50th percentile.

	Maternal Plasma PLP Levels		Cord Plasma PLP Levels	
	Upper 50% (43.2±21.0 pmol/ml)	Lower 50% (9.6±5.1 pmol/ml)	Upper 50% (160.4±44.3 pmol/ml)	Lower 50% (70.9±33.4 pmol/ml)
Birth weight (g)	3325±489 (n=11)	3461±324 (n=11)	3255±420 (n=10)	3548±387 (n=11)
Birth length (cm)	49.1±5.9 (n=7)	52.0±2.2 (n=10)	49.4±6.3 (n=8)	52.5±3.0 (n=9)
Placenta weight (g)	---	645.0±132.0 (n=5)	---	599.0±165.1 (n=5)
Apgar score, 1 min	8.3±0.6 (n=11)	7.4±2.2 (n=11)	8.5±0.5 (n=10)	7.2±2.3 (n=11)
Apgar score, 5 min	9.2±0.4 (n=11)	9.0±0.4 (n=11)	9.2±0.4 (n=10)	9.0±0.4 (n=11)

Means with asterisks are significantly different ($p < 0.01$).

Table 10. Number of infants receiving a given Apgar score at 1 minute after birth in each maternal vitamin B-6 supplementation group.

Apgar score	Vitamin B-6 Supplementation Level (mg/day)						
	0	2.6	5	7.5	10	15	20
1	0	1	1	0	0	0	0
6	0	2	0	0	0	0	0
7	1	1	0	1	0	0	1
8	1	2	3	1	1	1	1
9	5	4	2	5	3	5	3

pregnancy. No delivery samples were available for these subjects, although 30 week blood samples were obtained. However, these subjects were not included in statistical analyses involving the effect of vitamin B-6 supplementation since their compliance with the protocol was questionable. The 30-week plasma PLP levels for these women who were given 5, 10, and 15 mg pyridoxine-HCl supplements were 18.2, 73.1, and 60.6 pmol/ml, respectively. All three delivered normal-weight infants (>2500 g). The Apgar scores of the infant of only one mother were low. This subject had the lowest plasma PLP level at 30 weeks gestation of this group (18.2 pmol/ml) although her plasma PLP level at the initial visit was the highest (49.7 pmol/ml). The Apgar scores at 1 and 5 minutes after birth for her infant were 1 and 6.

Ten subjects of the original study volunteers were diagnosed as having pre-eclampsia at term. Blood samples were obtained at delivery from four of these subjects. Two subjects took the 20 mg pyridoxine-HCl supplement during pregnancy, and their maternal plasma PLP levels were 29.5 and 22.2 pmol/ml. The cord plasma PLP levels of these subjects were both 99.8 pmol/ml. These values are among the lowest in the 20 mg supplementation group. The other two subjects were given 12.5 mg supplements; but their compliance with the protocol was questionable, and they were therefore not included in the statistical analysis of the data. Both had low plasma PLP levels at delivery, and one was the lowest of any subject in the study. The maternal

plasma PLP levels at delivery were 2.4 and 19.4 pmol/ml, and the cord plasma PLP levels were 41.6 and 99.0 pmol/ml, respectively.

DISCUSSION

Nutrient Intake

The mean dietary vitamin B-6 intake of the subjects in this study (1.43 mg/day) was very similar to the intake estimated in a similar socioeconomic group of pregnant women attending the MIC clinic in Alachua county in 1978-1979 (200). The mean daily vitamin B-6 intake of that group was 1.37 ± 1.02 mg which was calculated by computer using a data base different from the one in this study. The mean dietary vitamin B-6 intake estimated in this study was also similar to values reported for men and women (236), women (136), female adolescents (237), and pregnant women (159, 200). Although the RDA for vitamin B-6 is increased during pregnancy, these data suggest that women do not increase their vitamin B-6 intake significantly during pregnancy.

The mean dietary vitamin B-6 intake was only 55% of the RDA for pregnant women while energy intake was close to the RDA and protein intake exceeded the RDA. This suggests that nutrient density may be a problem in the dietary choices of this population group. To meet the RDA for vitamin B-6 and energy, pregnant women must consume 1.13 mg of vitamin B-6 per 1000 kcal. In the present study pregnant subjects

consumed only 0.67 ± 0.63 mg/1000 kcal (mean \pm SD). When expressed as a ratio of vitamin B-6 to protein intake, the mean vitamin B-6 intake was 18.9 μ g/g protein. This intake meets 54% of the RDA when it is expressed as a ratio of the RDAs for vitamin B-6 and protein during pregnancy. Based on the same findings of vitamin B-6 depletion/repletion studies used to set the RDA for men and young women in the United States, the Dietary Standard for Canada recommends an intake of 20 μ g of vitamin B-6 per gram of dietary protein (112). This value would also apply during pregnancy since the increase in the RDA during pregnancy is to allow for the increased protein RDA. On this basis, the mean vitamin B-6 intake of the subjects in this study met about 95% of the recommended allowance.

Biochemical Indicators of Vitamin B-6 status

The data in this study demonstrated that maternal plasma PLP levels increased linearly with increasing amounts of vitamin B-6 supplementation. This linear relationship between plasma PLP and vitamin B-6 intake was previously demonstrated in rats fed 0, 4, 12, 24, and 100 μ g/day of pyridoxine (163). Rat skeletal muscle PLP also increased linearly with pyridoxine intake while liver and brain PLP levels reached a maximum when intake was 12 μ g/day or greater. Thus, plasma PLP functions as a storage and transport pool of this coenzyme, and is therefore considered

to be a sensitive indicator of vitamin B-6 status. Although the effects of graded vitamin B-6 levels in humans have not been reported previously, plasma PLP levels increased six-fold when 6 human subjects were given 100 mg of pyridoxine supplements per day for 1 to 3 weeks (80). The human plasma binding capacity for PLP has been estimated to be about 800 $\mu\text{g/ml}$ (3.23 $\mu\text{mol/ml}$) primarily due to the albumin concentration (81). Therefore, it appears that only very large pharmacological doses would result in saturation of plasma PLP in the adult and yield a nonlinear dose response curve.

In this study there were no significant differences in erythrocyte AspAT activities and stimulation by exogenous PLP among the vitamin B-6 supplementation groups. However, when changes in these indicators between the initial visit and 30 weeks gestation was calculated for each individual, vitamin B-6 supplementation did significantly affect stimulation of AspAT activity by added PLP but not basal AspAT activity. Previous research has resulted in conflicting reports regarding the sensitivity of erythrocyte aminotransferase activity and stimulation by added PLP as indicators of vitamin B-6 status. Research involving humans indicated that the degree of stimulation by PLP added in vitro was a better indicator than basal AspAT or AlaAT activities (160-162). However, Lumeng et al. (163) reported that erythrocyte AspAT and AlaAT activities were fairly sensitive indicators of vitamin B-6 status of rats while

stimulation values were not. When the vitamin B-6 requirement of women using oral contraceptives was assessed, random fluctuations in AspAT activity and stimulation by PLP were observed during depletion and repletion with vitamin B-6 (165). The data in this study indicate that stimulation of erythrocyte AspAT activity by exogenous PLP was more sensitive to vitamin B-6 supplementation than basal AspAT activity. Erythrocyte AspAT activity and stimulation by added PLP did not correlate with vitamin B-6 supplementation level or plasma PLP. These findings are consistent with other reports involving pregnant subjects (113, 117, 170, 171). The aminotransferase activities and stimulation values appear to be more useful indicators of vitamin B-6 status when vitamin B-6 deficiency is relatively severe or pyridoxine supplementation is given (113, 163). This may be due in part to the strong binding of PLP to these enzymes which would result in undersaturation only in a relatively severe vitamin B-6 deficiency (163).

Vitamin B-6 Status at Initial Visit

The vitamin B-6 status as measured by plasma PLP levels and stimulation of erythrocyte AspAT activity by added PLP of the pregnant subjects at the initial prenatal clinic visit was significantly lower than the nonpregnant comparison group. These findings confirm numerous reports that plasma PLP levels and coenzyme saturation of erythrocyte

aminotransferases are significantly decreased in pregnancy (32, 33, 113, 114, 116, 117). Although the mean plasma PLP level for the 196 subjects was not abnormally low which was defined as 2 standard deviations below the mean of the nonpregnant group (31.5 pmol/ml), 44% of the pregnant women did have plasma PLP levels below this value. Thus, almost half the women were already in suboptimum vitamin B-6 status at the initial prenatal appointment when the mean stage of pregnancy was 15 weeks. The mean gestational age of subjects with low plasma PLP levels (<31.5 pmol/ml) was about 2 weeks higher than those with adequate levels. This finding may be related to the hemodilution which occurs during pregnancy. Maternal plasma volume increases from about the tenth week to the 32nd week of pregnancy (209). However, plasma PLP levels did not correlate with the gestational age of these subjects which ranged from 6 to 21 weeks. This may have been due to such factors as the vitamin B-6 status of the subjects prior to pregnancy.

Various factors could affect vitamin B-6 status of pregnant women. Multiple parity theoretically could lead to depletion of maternal vitamin B-6 stores, particularly if the pregnancies were closely spaced. Since 75% of the subjects had only 2 or less previous pregnancies, it is not surprising that parity had no effect on indicators of vitamin B-6 status at the initial visit. The effect of race on measurements of vitamin B-6 status was investigated because racial differences in erythrocyte pyridoxal kinase

activities have been reported (89, 90). There were no racial differences in indicators of vitamin B-6 status in these subjects which supports previous reports that pyridoxal kinase is not an important regulator of PLP levels in erythrocytes under normal conditions (91). Chronic alcohol abuse has been shown to alter vitamin B-6 metabolism which results in decreased plasma PLP levels (129). Since alcohol use during pregnancy also has been reported to adversely affect pregnancy outcome, the effect of alcohol use during pregnancy on vitamin B-6 status throughout pregnancy as well as on the condition of the infant at birth was investigated. Alcohol use had no effect on vitamin B-6 status determined at the prenatal visit. Only 15% of the subjects indicated that they consumed alcohol during the pregnancy, and none admitted to chronic alcohol abuse.

Effect of Vitamin B-6 Supplementation on Vitamin B-6 Status

The data indicated that 5 mg of supplemental pyridoxine-HCl were able to maintain plasma PLP levels at 30 weeks gestation at values comparable to those found at the initial visit (figure 9). This same effect was observed when individual changes in the biochemical indicators of vitamin B-6 status were measured. As shown in figure 10, percent stimulation of erythrocyte AspAT activity by added PLP decreased between the initial visit and 30 weeks gestation when 5 mg of pyridoxine-HCl or more were taken. The percent

change in plasma PLP between the initial visit and 30 weeks gestation increased when supplementation was 5 mg or more (figure 11). However, 7.5 mg were required to prevent maternal plasma PLP levels at delivery from falling below initial levels (figures 9 and 11). This amount of maternal vitamin B-6 supplementation also appeared to saturate the fetal-placental system since cord plasma PLP levels reached a maximum at 7.5 mg of pyridoxine-HCl or greater (figure 7).

This suggests that PLP binding sites of fetal-placental unit such as albumin and vitamin B-6 dependent enzymes are saturated at about 7.5 mg supplemental pyridoxine-HCl or 7.6 mg total maternal vitamin B-6 intake (diet plus supplement as pyridoxine equivalents). Whether this saturation occurs at the placenta or fetus is not clear. Very little is known about the mechanism of vitamin B-6 placental transfer. PLP appears to be actively transported across the placenta since fetal PLP levels are much greater than maternal levels (32). However, increased circulating binding sites in the fetus may be involved in this phenomenon (33). Pyridoxal kinase in the placenta, present in relatively low amounts, may contribute some PLP to the fetal circulation (166). During early pregnancy the fetus is thought to lack the ability to phosphorylate vitamin B-6 vitamers. Fetal pyridoxal kinase activity in the liver and kidney has been positively correlated with gestational age which suggests that this increase in activity is in preparation for the newborn to carry out its own phosphorylation (32). The fetal

contribution to fetal plasma PLP levels through vitamin B-6 phosphorylation is unknown. The PLP and total vitamin B-6 content of the normal human placenta is much lower than other adult human tissues (w/w basis) (32). In a recent study in which women were given 20 mg of pyridoxine in addition to other water-soluble vitamins orally or intramuscularly during labor there was a 3-fold increase in total vitamin B-6 placental levels measured at delivery above that observed in nontreated subjects (238). The authors postulated that the placenta sequesters vitamins and that the placenta vitamin receptors must be saturated before adequate transfer of the vitamins to the fetus occurs. However, data was presented in only 3 subjects in each of the treatment groups which were matched for delivery times, and no statistical analysis was reported.

The data in the present study suggest that the maternal drain of plasma PLP at delivery is primarily due to fetal sequestration, and that this depletion can be prevented by saturating the fetal-placental system with 5 - 7.5 mg of supplemental pyridoxine-HCl or 5.5 - 7.6 mg total vitamin B-6 maternal intake (diet plus supplement as pyridoxine equivalents). The difference in apparent vitamin B-6 requirement between 30 weeks gestation and delivery indicates that the maternal vitamin B-6 requirement is higher during the last 10 weeks of pregnancy and that fetal sequestration of the vitamin is most active during this period. This observation is consistent with the fact that

the greatest fetal growth occurs during the last 10 weeks of pregnancy.

It is not known what level of cord plasma PLP is adequate or normal. For example, the plasma PLP levels of infants experiencing vitamin B-6 responsive convulsions have not been measured. The highest mean cord plasma PLP level measured in this study was 186 pmol/ml which occurred in the 10 mg supplementation group. Increased levels of supplementation up to 20 mg did not result in greater fetal plasma PLP levels. These data indicate that the circulating plasma PLP of the fetus is saturated when maternal vitamin B-6 supplementation is between 5 and 7.5 mg of pyridoxine HCl. However, maternal plasma PLP did not become saturated with the vitamin B-6 supplementation levels included in this study. These findings suggest that regulation of vitamin B-6 metabolism in the fetus may differ from maternal vitamin B-6 metabolism. Animal studies are required to investigate fetal vitamin B-6 metabolism.

Effect of Vitamin B-6 Supplementation and Status on Birth Outcome

The measurements of birth outcome in this study included birth weight, birth length, placental weight, and Apgar scores at 1' and 5 minutes after birth. The Apgar score is a rapid and simple method for evaluating the condition of newborn infants. A score of 0 to 2 points for each of the following signs for a maximum score of 10: heart rate, respiratory effort, muscle tone, reflex irritability, and

color (203, 204). The Apgar score at 1 minute after birth has been correlated with the clinical behavior of the infant during the first few hours of life, and infant mortality was inversely related to Apgar scores at 1 minute (203). It has been reported that the longer the Apgar score remains low, the worse the prognosis for survival (204). A good score at 1 minute after birth (8 - 10) rarely decreases when scoring is subsequently repeated (204).

The infants of mothers taking 7.5 mg or more supplemental pyridoxine-HCl in the present study had significantly higher Apgar scores at 1 minute after birth than infants whose mothers took 5 mg or less. In addition, infant Apgar scores at 1 minute were significantly higher when cord and maternal plasma PLP levels at delivery were above the 50th percentile than when these measurements were below the 50th percentile. These findings indicate that in this study birth outcome was associated with vitamin B-6 status. These data also indicate a possible favorable clinical effect of supplementation with 7.5 mg/day of pyridoxine-HCl or more on the infant condition at birth as measured by Apgar scores 1 minute after birth.

Condition of the infant at birth has previously been related to vitamin B-6 status of the mother. Roepke and Kirksey (159) reported that the maternal serum vitamin B-6 levels were lower in mothers whose infants had Apgar scores less than 7 at 1 minute after birth than in those whose infants scored 7 or better. Schuster et al. (200) found that

infants whose mothers exhibited poor vitamin B-6 status at about 15 weeks gestation had significantly lower Apgar scores at 1 minute after birth than those whose mothers exhibited adequate vitamin B-6 status as measured by in vitro stimulation of AlaAT activity by PLP.

Recently, Kirksey et al. (239) reported that the breast-fed infant of a mother receiving 5 mg of vitamin B-6 during pregnancy developed seizures by 7 days postpartum. The plasma PLP levels of the mother were very low at delivery (13.7 pmol/ml). The cord plasma PLP level was 82.0 pmol/ml which had decreased to 11.3 pmol/ml in the infant by 7 days postpartum. When maternal supplementation was increased to 20 mg/day, the infant became asymptomatic without additional medication. These data suggest that inadequate maternal vitamin B-6 intake may contribute to neurological abnormalities in the infant and/or fetus. Animal studies have demonstrated that inadequate maternal vitamin B-6 intake result in decreased litter weights (142, 191) and in delayed and impaired neuromotor development of the offspring (142, 143).

Vitamin B-6 Requirement during Pregnancy

The vitamin B-6 requirement of pregnant women is not known and has not been fully investigated. This is primarily due to the ethical considerations involved in performing traditional vitamin requirement studies with pregnant women.

The vitamin B-6 requirement of men and young women has been determined by depletion of body stores for a period of time and then repletion by dietary vitamin B-6 until predepletion levels of the biochemical indicators of vitamin B-6 status are reached (103-111). The current RDA for vitamin B-6 during pregnancy is based on the requirement determined for young women plus additional vitamin B-6 required for the metabolism of the increased protein allowance during pregnancy (100). The primary objective of this study was to provide a more realistic estimate of the vitamin B-6 requirement of pregnant women in view of the mounting data that biochemical indicators of maternal vitamin B-6 during pregnancy were very low, particularly at term, in a significant number of women (32, 33, 113, 114, 117). The experimental design of this study allowed the vitamin B-6 requirement of pregnant women to be estimated without compromising maternal or fetal health by assessing the effect of graded levels of vitamin B-6 supplementation on maternal and fetal vitamin B-6 status and on birth outcome.

Although it is not known whether low maternal plasma PLP levels during pregnancy and at term relative to nonpregnant women actually constitute a vitamin B-6 deficiency, the following points support the desirability of maintaining the often already low plasma PLP levels found earlier in pregnancy. The decrease in plasma PLP levels cannot be adequately accounted for by the increase in maternal plasma volume which occurs in pregnancy beginning

at the end of the third month. The most rapid rate of decrease in plasma PLP occurs between the 30th and 40th weeks (term) of pregnancy after the greatest increase in plasma volume has already occurred (209). The greatest growth of the fetus and the placenta also occurs between the 30th week of pregnancy and term, and it is important to keep in mind that the placenta also has its own nutritional requirements (209). The growth rate of the fetal brain is most rapid just before birth, and PLP is involved in myelination and other aspects of the development of the fetal nervous system (140, 149, 151).

In 1978 the American Academy of Pediatrics (240) recommended that human milk be the sole source of nutrients for infants during the first 4 to 6 months. Since maternal vitamin B-6 blood levels have been shown to affect directly the vitamin B-6 content of the milk, mothers beginning lactation with low plasma PLP levels at term may adversely affect the vitamin B-6 status of their breast-fed infants. Kirksey et al. (206) reported the vitamin B-6 intake of breast-fed infants of subjects who took no supplemental vitamin B-6 ranged from 40 to 50 $\mu\text{g}/\text{day}$. Bessey et al. (139) reported that infants require 200 $\mu\text{g}/\text{day}$ and that levels below 85 $\mu\text{g}/\text{day}$ have resulted in grand mal seizures. When lactating mothers were supplemented with 2.5, 10 or 20 mg/day, mean infant intakes were estimated to be 100, 190, and 430 $\mu\text{g}/\text{day}$, respectively (206). In view of the American Academy of Pediatrics recommendation, it would be prudent to

prevent the depletion of maternal plasma PLP reported to occur by term (32, 33, 117).

The data in this study have demonstrated that a maternal vitamin B-6 intake of about 5.5 to 7.6 mg/day (diet plus supplement as pyridoxine equivalents) maintains maternal plasma PLP levels at delivery comparable to those found at the initial prenatal clinic visit. This amount of maternal intake also appeared to saturate the plasma PLP of the fetal-placental unit. Infants of mothers consuming this amount or more were in significantly better condition at birth as measured by Apgar scores 1 minute after birth. Therefore, the vitamin B-6 requirement of pregnant women to ensure adequate maternal and fetal vitamin B-6 status at delivery appears to be between about 5.5 and 7.6 mg/day.

Vitamin B-6 Status and Morning Sickness

The data revealed no relationship between vitamin B-6 status of pregnant women at the first prenatal clinic visit and the degree of morning sickness experienced by these subjects during the first trimester of pregnancy. It has been reported that 24 women suffering from morning sickness had significantly lower plasma PLP levels than women without morning sickness symptoms and that vitamin B-6 supplementation relieved the severity of these symptoms (216). However, the data in this study from almost 180 pregnant women demonstrated that there were no differences

in the biochemical indicators of vitamin B-6 status between women who experienced morning sickness and those who did not. The findings of this study do not rule out the possibility that pyridoxine administration may alleviate morning sickness symptoms. However, if pyridoxine is effective in treating morning sickness, the data in the present study indicate that its efficacy is not related to inadequate vitamin B-6 status of the pregnant woman.

The only significant differences between the two groups were in parity and placental weights. Women who experienced morning sickness had a greater number of previous pregnancies and greater placental weights than those who did not. There are several reports that morning sickness is a favorable prognostic sign of pregnancy. Women with morning sickness have a decreased risk of abortions prior to the 20th week of pregnancy (241-243) and give birth to infants with higher birth weights (241).

Vitamin B-6 and Pre-eclampsia

The maternal and cord plasma PLP levels of the 4 subjects who developed pre-eclampsia in this study were relatively low. The placental uptake of other nutrients which involve active transport such as amino acids has been shown to be decreased in pre-eclampsia (225). It is possible that pre-eclampsia may also adversely affect the fetal uptake of PLP, and this may be one factor in the low cord

plasma PLP levels found in this study and reported by others (201, 223, 224). Other contributing factors may be the low levels of pyridoxal kinase and PMP oxidase in pre-eclamptic placentae compared with normal placentae (223).

CONCLUSIONS

The objectives of this study were to estimate the vitamin B-6 requirement of pregnant women by assessing the effects of graded levels of vitamin B-6 supplementation on vitamin B-6 status of the mother and the fetus. In addition, the effect of vitamin B-6 status throughout pregnancy on the outcome of pregnancy and on the condition of the infant at birth were evaluated. The relationships between morning sickness of pregnancy, pre-eclampsia, and gestational diabetes, and vitamin B-6 status in this population group were also investigated.

The findings of this study indicate that maternal intake between approximately 5.5 and 7.6 mg of vitamin B-6 (between 5 and 7.5 mg of pyridoxine-HCl plus dietary intake) appears to saturate the fetal-placental system plasma PLP levels while concurrently preventing a decrease in maternal plasma PLP levels at delivery below the levels found early in pregnancy. Such an intake exceeds the current RDA and would necessitate supplementing pregnant women with vitamin B-6 since it would be extremely difficult to obtain that amount from the diet alone. However, this step may prove desirable since the condition of the infants of those subjects which took 7.5 or more supplemental pyridoxine-HCl was significantly better as measured by Apgar scores 1

minute after birth. This is also important in the lactating mother since inadequate maternal vitamin B-6 status at term could seriously compromise the vitamin B-6 status of her breast-fed infant.

No relationship was found between the degree of morning sickness and vitamin B-6 status in early pregnancy in this population group. However, women who did experience morning sickness had a greater number of previous pregnancies and greater placental weights than those who did not.

The Committee on Maternal Nutrition of the Food and Nutrition Board of the National Research Council (209) in 1970 cited the need for information to establish desirable levels of nutrients in maternal blood and to determine factors which control these levels and subsequent transport of nutrients across the placenta. Since then, little information about vitamin B-6 in this regard has been forthcoming. In the present study the amount of maternal vitamin B-6 intake during pregnancy which saturates the fetal-placental system and maintains maternal plasma PLP at a level comparable with that found in early pregnancy has been estimated. These data have also demonstrated that the condition of infants whose mothers consumed this amount or more was significantly better than infants of mothers who consumed less. Therefore, it is hypothesized that this estimate represents the approximate vitamin B-6 requirement of the pregnant woman to ensure adequate vitamin B-6 status and health of the infant at birth. Further research is

APPENDIX

Form 1

Vitamin B-6 Study Description and Instructions

You are invited to participate in a study which will help nutritionists find out what amount of vitamin B-6 is best for pregnant women.

If you volunteer, you will be given a bottle of vitamin B-6 supplements during your first visit to the Maternal and Infant Care clinic. It is very important that you take one of these vitamin tablets every day. If for some reason you forget to take the vitamin tablet, you may go ahead and take it the next day along with the tablet for that day.

At around your 30th week of pregnancy, you will have a blood sugar test at the clinic. Be sure you bring your bottle of vitamin B-6 supplements on that clinic visit. You will be given a new bottle of supplements at that time to take until you deliver your baby. Also at that visit, a nutritionist will record your food intake of the day before.

Your blood is normally taken as part of your prenatal care at your first visit to the clinic and the 30th week

visit. At that time a small extra amount of blood will be taken for this study. An extra sample of blood from you and the cord will be taken at delivery.

You are free to withdraw from the study at any time. Your nutritionist at the clinic will answer any questions you have about the study. Information about the effect of your vitamin B-6 supplement on your blood levels and how nutritious your diet is will be sent to you upon request.

Form 2

Informed Consent

Subject Name: _____

Project Title: Vitamin B-6 Requirement Determination in Pregnant Women.

You are invited to participate in a nutrition study designed to determine how much vitamin B-6 (a water-soluble vitamin) should be taken daily by pregnant women. The amount of vitamin B-6 in your blood after a supplement is taken will be determined during the study. Your diet will be analyzed by computer to determine what quantity of vitamins and minerals you consume daily.

If you would like to volunteer for the study, you will:

1. Be assigned a supplement to take daily. The supplement will contain either varying amounts of vitamin or no vitamin B-6 if you receive a control supplement.

2. Have an additional sample of your blood drawn at the same time your routine clinic samples are drawn.

3. Have an extra sample of your blood and cord blood collected following delivery.

4. Have information regarding pregnancy outcome and the condition of your infant at birth recorded from your clinic records.

5. Complete a diet history form.

If you participate in the study, you may receive information about how nutritious your diet is and what effect your supplement had on your vitamin B-6 blood levels. All questions you may have can be answered by the clinic nutritionist. You are free to withdraw your consent and to discontinue participation in the project at any time without prejudice. Vitamins will not be withheld from you because of participation in this study. You will not be cautioned against taking vitamins solely for this study. Appropriate vitamin B-6 therapy will be recommended if your vitamin B-6 levels are determined to be below "normal" during this study.

All information will be confidential to the extent provided by law. "In the event of my sustaining a physical injury which is proximately caused by this experiment, professional medical care received at the J. Hillis Miller Health Center exclusive of hospital expenses will be provided me without charge."

I have fully explained to _____ the nature and purpose of the above-described procedure and the risks that are involved in its performance. I have answered and will answer all questions to the best of my ability.

Name & signature of person
obtaining consent

Date

I have been fully informed of the above-described procedure with its possible benefits and risks and have received a copy of this description. I have given permission for my participation in this study.

I understand that I am free to withdraw this consent and discontinue participation in this project at any time without its affecting my care.

Signature of subject

Witness to signature

Date

Principal Investigator

Date

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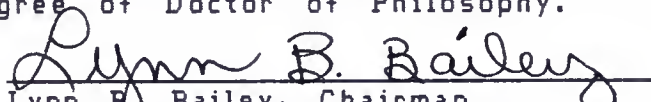
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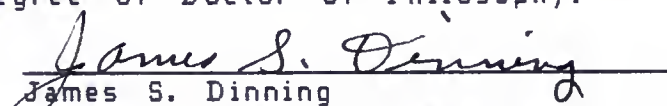
BIOGRAPHICAL SKETCH

Karen Ann (Thurston) Schuster was born in Nurnberg, Germany, in 1950. She moved to Indianapolis, Indiana, with her parents in 1957 where she attended school until high school graduation in 1968. She attended Indiana University for one year and worked in Florida until moving to Germany in 1972. She met Glenn Russell Schuster in Germany, and they were married in 1974. Karen and Glenn made their home and continued their education in Hilo, Hawaii, where Karen graduated from the University of Hawaii at Hilo with high honors in chemistry. They moved to Gainesville in 1978 to pursue graduate studies at the University of Florida, and Karen received a Master of Science degree from the Department of Food Science and Human Nutrition in 1980. The American Institute of Nutrition awarded Karen a Graduate Student Research Award in 1980 for her master's research. She expects to receive a Doctor of Philosophy degree in August 1983.

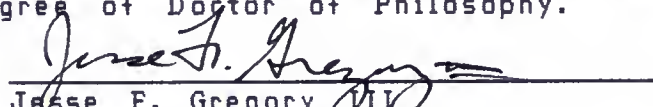
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Lynn B. Bailey, Chairman
Associate Professor of Food
Science and Human Nutrition

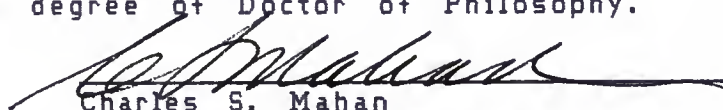
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James S. Dinning
Research Scientist, Department of
Food Science and Human Nutrition

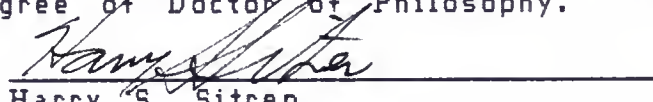
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Jesse F. Gregory
Associate Professor of Food
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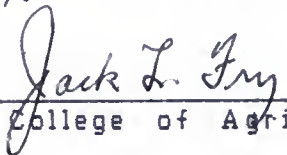

Charles S. Mahan
Professor of Obstetrics and
Gynecology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Harry S. Sitren
Assistant Professor of Food
Science and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1983



Dean, College of Agriculture

Dean for Graduate Studies and
Research

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